"A COMPARATIVE STUDY ON SALIVARY TUMOR NECROSIS FACTOR-ALPHA LEVELS IN NON-SMOKERS AND SMOKERS WITH CHRONIC PERIODONTITIS BEFORE AND AFTER PHASE-I PERIODONTAL THERAPY"

A Dissertation submitted in
partial fulfillment of the requirements
for the degree of

MASTER OF DENTAL SURGERY

BRANCH – II

PERIODONTOLOGY

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI – 600 032

2016 – 2019

CERTIFICATE BY HEAD OF THE DEPARTMENT /

HEAD OF THE INSTITUTION

This is to certify that the Dissertation entitled **"A COMPARATIVE STUDY ON SALIVARY TUMOR NECROSIS FACTOR-ALPHA LEVELS IN NON-SMOKERS AND SMOKERS WITH CHRONIC PERIODONTITIS BEFORE AND AFTER PHASE-I PERIODONTAL THERAPY"** is a bonafide work done by **Dr. AADHIRAI. M**, Post Graduate student (2016–2019) in the Department of Periodontology, under the guidance of **Dr. K. MALATHI, M.D.S**, Head of the Department, Department of Periodontics, Tamil Nadu Government Dental College and Hospital, Chennai – 600 003.

Dr. K. MALATHI, M.D.S,
Head of the Department & Guide,
Department of Periodontology.

Dr. G.VIMALA, M.D.S,
Principal,
TNGDC&H.

TAMIL NADU GOVERNMENT DENTAL COLLEGE AND HOSPITAL

CHENNAI – 600 003

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This is to certify that **Dr. AADHIRAI. M**, Post Graduate student (2016–2019) in the Department of Periodontology, Tamil Nadu Government Dental College and Hospital, Chennai – 600 003 has done this dissertation titled **"A COMPARATIVE STUDY ON SALIVARY TUMOR NECROSIS FACTOR-ALPHA LEVELS IN NON-SMOKERS AND SMOKERS WITH CHRONIC PERIODONTITIS BEFORE AND AFTER PHASE-I PERIODONTAL THERAPY"** under my direct guidance and supervision in partial fulfillment of the regulations laid down by **TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY**, Chennai – 600 032 for **M.D.S,** Branch – II **PERIODONTOLOGY** degree examination.

Dr. K. MALATHI, M.D.S,

Head of the Department and Guide

Department of Periodontology

TAMIL NADU GOVERNMENT DENTAL COLLEGE AND HOSPITAL

CHENNAI – 600 003

DECLARATION BY THE CANDIDATE

TITLE OF STUDY	A COMPARATIVE STUDY ON SALIVARY TUMOR NECROSIS FACTOR-ALPHA LEVELS IN NON-SMOKERS AND SMOKERS WITH CHRONIC PERIODONTITIS BEFORE AND AFTER PHASE-I PERIODONTAL THERAPY.
PLACE OF STUDY	TAMIL NADU GOVERNMENT DENTAL COLLEGE AND HOSPITAL, CHENNAI – 600 003.
DURATION OF THE COURSE	3 YEARS
NAME OF THE GUIDE	Dr. K. MALATHI, M.D.S
HEAD OF THE DEPARTMENT	Dr. K. MALATHI, M.D.S

I hereby declare that this dissertation titled **"A COMPARATIVE STUDY ON SALIVARY TUMOR NECROSIS FACTOR-ALPHA LEVELS IN NON-SMOKERS AND SMOKERS WITH CHRONIC PERIODONTITIS BEFORE AND AFTER PHASE-I PERIODONTAL THERAPY"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. K. MALATHI, M.D.S,** Head of the Department and Guide, Department of Periodontology, TamilNadu Government Dental College and Hospital, Chennai -600003.

Dr. K. MALATHI, M.D.S — **HOD & Guide**

Dr. G. VIMALA, M.D.S — **Principal**

Dr. AADHIRAI. M — **Student**

TAMIL NADU GOVERNMENT DENTAL COLLEGE AND HOSPITAL

CHENNAI – 600 003

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Above all, I thank ***GOD ALMIGHTY*** *to have been showering his blessings throughout and to have given all these wonderful people in my life.*

TRIPARTITE AGREEMENT

This agreement herein after the "Agreement" is entered into on this day ---------- between the Tamil Nadu Government Dental College and Hospital represented by its **Principal** having address at Tamil Nadu Government Dental College and Hospital, Chennai-600 003, (hereafter referred to as, 'the college')

And

Dr. AADHIRAI. M, aged 26 years currently studying as **Post Graduate student** in the Department of Periodontology, Tamil Nadu Government Dental College and Hospital, Chennai-600 003 (hereafter referred to as 'the PG student and principal investigator')

And

Mrs. Dr. K. MALATHI, M.D.S, aged 54 years working as **Head of the Department and Professor** in the Department of Periodontology, Tamil Nadu Government Dental College and Hospital, Chennai-600003 (herein after referred to as the 'Co-Investigator')

Whereas the PG student as part of her curriculum undertakes this research on **"A COMPARATIVE STUDY ON SALIVARY TUMOR NECROSIS FACTOR-ALPHA LEVELS IN NON-SMOKERS AND SMOKERS WITH CHRONIC PERIODONTITIS BEFORE AND AFTER PHASE-I PERIODONTAL THERAPY"** for which purpose the Co-investigator and the college shall provide the requisite infrastructure based on availability and also provide facility to the PG student as to the extent possible as a principal investigator.

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TELEPHONE : 044-253403343
FAX: 044- 25300681

Ref No: R. C. NO: 0420/DE/2016
date : 08-11-2016

Sub: IEC review of the research proposals,

Title of the work: **A comparative study on salivary Tumour Necrosis Factor-Alpha levels in nonsmokers and smokers with chronic periodontitis before and after phase-I periodontal therapy.**

Investigator: **Dr. M.AADHIRAI**
I year , MDS

Department : **Department of Periodontics**
Tamil Nadu Govt. Dental College & Hospital ,

Thank you for submitting your research proposal , which was considered at the Institutional Ethics Committee meeting held on the 01-11-2016, at TN Govt. Dental College. The documents related to the study referred above were discussed and reviewed.

The decision of the members of the committee , the secretary and the Chairperson IEC of TN Govt. Dental College is here under:

Approved	Approved and advised to proceed with the study
Approved with suggestions	----------

The principal investigators and their team are advised to adhere to the guide lines given below:

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1. You should carry out the work without affecting regular work and without extra expenditure to the Institution or the Government.
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ABSTRACT

BACKGROUND: Tumor Necrosis Factor-alpha (TNF-α) is one of the pro-inflammatory cytokines that has been considered to be involved in the pathogenesis of periodontal disease as mediators of tissue destruction and serves as a biomarker for disease progression. Increased level of TNF-α has been identified in oral fluids of smokers. Recent advances have enabled the use of saliva as diagnostic fluid to detect the levels of these biomarkers.

AIM: The aim of the present study is to compare the level of TNF-α in saliva of non-smokers and smokers with chronic periodontitis before and after phase-I therapy.

MATERIALS AND METHOD: Level of salivary TNF-α was determined in non-smokers with chronic periodontitis (control group, n=15) and smokers with chronic periodontitis (study group, n=15) and compared with clinical parameters like gingival bleeding index, plaque index, probing pocket depth and clinical attachment level before and after phase-I therapy.

RESULTS: Smokers had increased level of Salivary TNF-α compared to non-smokers at baseline. Statistically significant (p=0.00) reduction in clinical parameters and Salivary TNF-α level were seen among both non-smokers and smokers 3 months after Phase-I therapy. However, non-smokers showed significantly greater reduction in clinical parameters and Salivary TNF-α level compared to smokers.

CONCLUSION: The present observations suggest that smoking is associated with elevated level of salivary TNF-α level and non-smokers exhibit better response to Phase-I therapy which is evident through significant decrease in level of salivary TNF-α after phase-I therapy compared to smokers.

KEY WORDS: Smoking, TNF-α, Phase-I Therapy, Chronic Periodontitis

CONTENTS

LIST OF PHOTOGRAPHS

LIST OF TABLES

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AP-1	**Activator Protein -1**
CAL	**Clinical Attachment Level**
CCL	**Chemokine Ligand**
CD	**Cluster Differentiation**
CEJ	**Cemento Enamel Junction**
COX	**Cyclooxygenase**
CXCR3	**Chemokine receptor**
DED	**Death Effector Domain**
DNA	**Deoxyribo Nucleic Acid**
EC	**Endothelial cell**
ELISA	**Enzyme Linked Immunosorbent Assay**
FADD	**Fas Associated protein with Death Domain**
GCF	**Gingival Crevicular Fluid**
HGF	**Human Gingival Fibroblast**
ICAM	**Intercellular Adhesion Molecule**
ICTP	**Collagen Telopeptide I**
IFN	**Interferon**
IL	**Interleukin**
JAK-STAT	**Janus kinase/ signal transducers and activators of transcription**
JNK	**Jun N – terminal kinase**
LIF	**Leukemia Inhibitory Factor**
LPS	**Lipopolysaccharide**
MAPK	**Mitogen Activated Protein kinase**

MMP	**Matrix Metallo Proteinase**
NF-KB	**Nuclear Factor - KB**
NLR	**Nucleotide binding domain, leucine rich containing protein**
OD	**Optical Density**
OPG	**Osteoprotegerin**
PD	**Periodontal Disease**
PGE	**Prostaglandin E**
PGF2	**Prostaglandin F2**
PMN	**Polymorphonuclear Neutrophil**
PPD	**Probing Pocket Depth**
RANK	**Receptor Activated Nuclear Factor Kappa-B**
RANKL	**Receptor Activated Nuclear Factor Kappa-B Ligand**
RANTES	**Regulated on activation, normal T cell expressed and secreted**
rpm	**Rotations per minute**
sICAM	**Soluble Intercellular Adhesion Molecule**
SPSS	**Statistical Package for Social Science**
TGF	**Transforming Growth Factor**
Th	**T helper cell**
TLR	**Toll Like Receptor**
TNFR	**Tumour Necrosis Factor Receptor**
TNF-α	**Tumour Necrosis Factor-α**
VCAM	**Vascular Cell Adhesion Molecule**
VEGF	**Vascular Endothelial Growth Factor**

INTRODUCTION

Periodontitis is a chronic bacterial infection characterized by persistent inflammation, connective tissue breakdown and alveolar bone destruction. Microbial antigens present within the periodontal tissue initiate an inflammatory response in the host. The tissue destruction and disease progression that occurs in periodontal diseases is related to a prolonged but unprotected host response which may be mediated by genetic, environmental and systemic factors. This host response leads to a prolonged release of inflammatory mediators like pro-inflammatory cytokines, kinins and eicosanoids[1].

Proinflammatory cytokines are cytokines that are important in cell signalling, promote systemic inflammation and are involved in the up regulation of inflammatory reactions. They are linked to extensive tissue destruction while anti-inflammatory cytokines counteract and attenuate disease progression. The imbalance between the pro inflammatory and anti-inflammatory cytokines may be an important determinant of periodontal disease progression.

Tumor necrosis factor-alpha (TNF-α) is one such pro-inflammatory cytokine which has a myriad of actions including facilitation of leukocyte recruitment to the site of inflammation, altering vascular permeability by stimulated expressions of selectins and adhesins, inducing synthesis of interleukins and prostaglandins and vascular proliferation in the formation of periodontal granulation tissue[2]. Prolonged production of TNF-α results in excess bone resorption as it enhances osteoclastogenesis by up regulating RANK-RANKL coupling mechanism and by increasing the production of other pro-inflammatory cytokines like IL-1, 6 and 8[3].

Smoking has been found to exert a major effect on the protective elements of immune response, thereby increasing extent and severity of periodontal destruction. However, smokers and non-smokers with periodontal disease largely exhibit a similar micro flora in terms of the periopathogens[4]. Smoking alters the host response, including vascular function, neutrophil/monocyte activities, adhesion molecule expression, antibody production, as well as cytokine and inflammatory mediator release[5]. These changes likely contribute to the negative impact of smoking on the reparative and regenerative potential of the periodontium.

Treatment of periodontal disease includes both non-surgical and surgical therapy. Non-surgical therapy, including scaling and root planing, remains the corner stone of periodontal treatment to control disease progression. There is a need for newer diagnostic tests that would help in early recognition of the microbial challenge, assessment of current disease activity, prediction of sites that are vulnerable for future breakdown and prediction of the periodontal therapy administered.

The localized nature and its proximity to periodontal lesions in the oral cavity, ready availability & ease in collection have increased the utilization of saliva as a natural biological fluid for measurement of microbial and protein biomarkers of the diseases process.

Therefore estimation salivary TNF-α level can be considered as a biomarker for diagnosis, evaluation of treatment response and prognosis of generalized chronic periodontitis.

AIM AND OBJECTIVES

AIM

The aim of this study is to compare the levels of the TNF-α in saliva of non-smokers and smokers with chronic periodontitis before and after phase-I therapy.

OBJECTIVES

For this purpose the following objectives were chosen:

- To measure clinical parameters like gingival bleeding index, plaque index, probing pocket depth and clinical attachment level for all the subjects before and after phase-I therapy.
- To estimate and compare salivary TNF-α levels of non-smokers and smokers with chronic periodontitis before and after phase-I therapy.
- To correlate salivary TNF-α levels of non-smokers and smokers with chronic periodontitis with clinical parameters like gingival bleeding index, plaque index, probing pocket depth and clinical attachment level before and after phase-I therapy.

REVIEW OF LITERATURE

CHRONIC PERIODONTITIS

The progression of gingivitis to periodontitis is a slow process that occurs as loss of attachment over a long period of time and also this results in rapid attachment loss that occurs in episodic burst in short time.[6] It is now evident that periodontal diseases in humans has heterogeneous aetiologies stemming from the development of biofilm in the subgingival environment social and behavioural factors and genetic or epigenetic traits, all of which are modulated and controlled by the underlying immune and inflammatory responses of the host.

As a result of biofilm maturation, the pathogenic species developing in the periodontal pockets release an array of virulence factors, antigens, or by products that evade the host defence mechanism, causing damage to cells and tissue via dysregulated inflammatory interactions, which typically consists of neutrophil, monocytes/macrophages, dentritic cells, T-cells and predominantly immunoglobulin producing B-cells[7]. The infected tissue/cells are overwhelmed by persistent pathogens accompanied with a lasting chronic inflammation where the potent pro inflammatory mediators and cytokines prevail. Inflammation is a characteristic feature that depicts the trigger of the defence mechanism against an invading pathogen in the body which is accompanied by a release of many cytokines.

Cytokines are low molecular protein involved in initiation and effector stages of immunity and inflammation, in which they regulate the amplitude and duration of the response. They are the cell regulators that have a major influence on the production and activation of different effector cells which decides the type of immune response

that occurs on exposure to a pathogen which is in turn is vital in determining resistance or susceptibility to diseases.

Balkwill et all (1989)[8] found that cytokines interact in a network firstly by inducing each other, secondly, transmodulating cell surface receptors and thirdly, by a synergistic, additive or antagonistic interaction on cell function.

Assuma et al (1998)[9] stated that cytokines interact with specific cell surface receptors which are usually expressed in relatively low numbers. Many cytokines are pleiotropic, having multiple activation on different target cells and or overlapping cell regulatory actions. But despite this overlap cytokine function may be identical.

Hosokawa et al (2005)[10] evidenced that cytokines played a critical role in a nonhuman primate model. In this report, inhibition of IL-1 and TNF-α reduced the progression of periodontal bone loss and loss of attachment, which was attributed to the recruitment of inflammatory cells (notably monocytes and lymphocytes) toward the bone.

Mahanonda R et al (2007)[11] suggested that a critical aspect of the host response is the detection of bacteria by Toll-like receptors (TLRs). Activation of the innate immune response by the binding of various bacterial components (i.e, diacyl lipopeptides, peptidoglycan, LPS, flagellin, and bacterial DNA) to TLRs results in the production of cytokines and chemokines. Upon activation of TLRs, an intracellular signalling cascade is stimulated that leads to the activation of transcription factors (e.g., nuclear factor-kappa B, activator protein 1 (AP-1) and p38) and the production of various cytokines, many of which directly or indirectly stimulate osteoclast formation.

Vandyke et al (2014)[12] explained that in susceptible individuals, dysregulation of inflammation and immune pathways led to chronic inflammation, tissue destruction and periodontal disease.

Meyle et al (2015)[13] suggested that host response is now recognized as major contributor to periodontal tissue damage in what becomes dysfunctional, poorly targeted unresolving inflammation that only serves to nourish and sustain dysbiosis.

Franco Cavalla et al (2018)[14] concluded that exacerbated and uncontrolled immune response are responsible for periodontal tissue destruction in chronic periodontitis and signalling molecules, especially cytokines, like IL-6 and TNF-α link the inflammatory response with uncoupling of bone metabolism that leads to periodontal bone destruction.

TUMOR NECROSIS FACTOR-α

A successful immune response to an infectious agent depends on activation of appropriate effector functions. As periodontitis is a chronic inflammatory disease, it is certainly reasonable that such a condition establishes a local cytokine environment that influences the immune response. It is becoming increasingly clear, however, that cytokines do not function in isolation, but rather in complex networks involving both pro- and anti-inflammatory effects. Production of appropriate cytokine is essential for the development of protective immunity. If inappropriate cytokines is elicited destructive or progressive disease can result (**Kelso et al 1990)**[15].

The extent of periodontal tissue destruction is mainly determined by the balance maintained between the pro- and anti- inflammatory cytokines and the regulation of their receptor and signalling pathways. Pro inflammatory cytokines mediate the tissue

damage, which leads to loss of function and clinical disease. The persistent activation of immune responses leads to increased synthesis and secretion of these pro inflammatory cytokines with concomitant effects on function and turnover of periodontal cells.

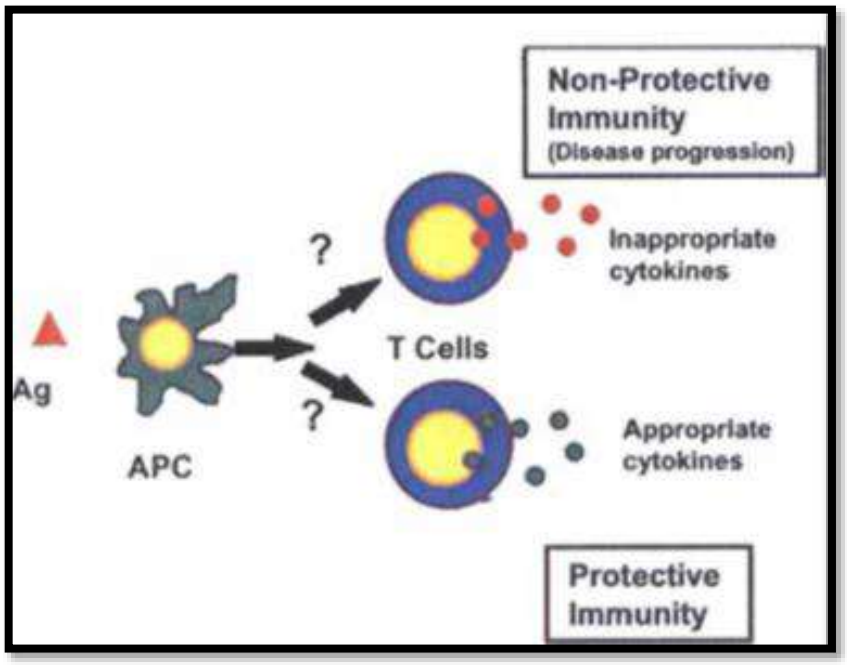


Fig 1: Release of protective and non-protective cytokine
(Courtesy: Journal of periodontology, 2002[16])

The importance of cytokines in the pathogenesis of periodontal disease is apparent at a number of levels. Not only do they act as initiators and regulators of innate and adaptive immunity but they also mediate the tissue damage, which leads to loss of function and clinical disease. Many of the non-immune cell types of the periodontium (e.g. keratinocytes and fibroblasts) synthesize cytokines in response to bacteria and other cytokines, and cytokines also influence turnover of extracellular matrix components and the fibres of the periodontal ligament[16].

Cytokines also have a central role in osteoclast activation. Cytokines drive the tissue destruction that results in the clinical manifestations of periodontitis through

myriad overlapping effects on cells and mediators in the periodontium. The complex interactions between cytokines and immune responses make it difficult to distinguish and compartmentalize different aspects of the role of cytokines in driving tissue destruction[17].

Cytokines up regulate the production of inflammatory mediators in the periodontium (e.g. prostaglandins, MMPs, cytokines, chemokines) leading to tissue destruction. Multiple feedback loops develop; for example, cytokines induce the secretion of prostaglandins, and increased prostaglandin concentrations result in increased cytokine secretion[18].

TNF-α is one among the pro-inflammatory cytokines that induces bone resorption and leads to up regulated production of PGE2 and MMP secretion.

Snyderman R et al (1982)[19] suggested that periodontitis has high levels of proinflammatory cytokines, including IL-1β and TNF-α and low levels of cytokines which suppress the immunoinflammatory response such as IL-10 and IL-4. These cytokines are associated with the active stages of periodontitis.

Clements et al (1991)[20] proposed that the TNF-α is produced mainly by macrophages in response to agents such as lipopolysaccharide. The decrease in TNF-α seemed to reduce the host response, thereby leading to higher levels of bacteria; however, because the host response was less, there was a reduced expression of the cytokines that stimulate bone resorption, which resulted in net bone loss.

Yucel-Lindberg (1995)[21] also found that TNF-α also induced IL-1a and IL-1b production in HGFs and this was synergistically enhanced by the presence of bradykinin.

Mosman et al (1996)[22] studied that Thl cytokines, especially TNF-α, are involved in cell mediated inflammatory reactions. They increase the ability of macrophages to kill intracellular and extracellular pathogens and also mediate delayed type hypersensitivity reactions.

Kent et al (1998)[23] studied that IL-1β and TNF-α act synergistically in stimulating IL-6 secretion by HGFs and this combination of cytokines was shown to be many hundreds of times more potent in stimulating IL-6 production than LPS.

Kobayashi and Okada et al (1999)[24] found that IL-1β and TNF-α induced IL-1α secretion in HGFs and this production was differentially modulated by T-cell derived cytokines including IFN-ɣ and IL-4.

Romagnani et al (2000)[25], has characterized 3 subsets of T-helper cells based on their cytokine profile. Typical secretory products of Th1 cells are IL-2. IL- 12, TNF-α and IFN-ɣ and those of Th2 cells are IL-4, IL-5, IL-6, IL-10 and Th3 are known to secrete TGF-β.

Liu et al (2001)[26] studied enhanced accumulation of PMNLs has been reported in the gingival tissues of patients with periodontitis, and has been associated with up regulated IL-8, ICAM-1, IL-1β and TNF-α expression.

Dinarello et al (2001)[27] found that the TNF-α plays a critical role in both innate and adaptive immune responses, up-regulating antigen presentation and the bactericidal activity of phagocytes.

In another study, **Noguchi et al (2002)**[28] found that the TNF-α, IL-1β and PGF2a all stimulated IL-6 production in cultured HGFs and PGF2 synergistically increased IL-6 production stimulated by TNF-α and IL- 1β.

Nakao et al (2002)[29] demonstrated that IL-1β and TNF-α synergistically increase PGE2 and TNF-α was shown to up regulate PGE2 and COX production in HGFs via the JNK and NF-kB signalling pathways.

Graves et al (2003)[18] showed that the TNF-α plays a central role in inflammatory reaction, alveolar bone resorption, and the loss of connective tissue attachment. He studied that IL-1β and TNF-α induce up regulation of adhesion molecules on leucocytes and endothelial cells, they stimulate the production of chemokines (which recruit circulating leucocytes to sites of inflammation) and they induce expression of other inflammatory mediators that potentiate inflammatory responses, such as the prostaglandins and MMPs.

Garlet et al (2007)[30] have demonstrated that similar cytokines considered harmful in the context of tissue destruction may play important roles in the control of periodontal infection.

Palmqvist et al (2008)[31] studied that there was a dose-dependent stimulation of IL-6 and LIF mRNA and protein by IL-1β and TNF-α and dose-dependent stimulation of IL-11 mRNA and protein by IL-1β.

Ohta et al (2008)[32] studied that Chemokine expression is stimulated by cytokines; for example, IL-1β and TNF-α increase the production of RANTES (CCL5) in HGFs and IFN-γ, TNF-α and IL-4 cooperatively regulate CXCR3 agonistic chemokines in oral keratinocytes and fibroblasts.

Kawai and Akaira et al (2010)[33] found that these cytokines (TNF-α and IL-1β) are synthesized by the activation of transcription factor NF-kB which is activated

by the recognition of LPS by a macromolecular complex involving CD14, MD-2 and TLR-4.

EFFECT OF TNF-α ON VASCULAR ENDOTHELIUM

Cytokines have increased effect on endothelial cells. Circulating cytokines interact with specific receptors on various cell types and activate JAK-STAT, NFKB & SMAD signalling pathway leading to an inflammatory response involving cell adhesion, permeability & apoptosis. Cytokine induced activation of these pathways in endothelial cells modifies the production/activity of vasodilator mediators like nitric oxide, prostacyclin, endothelium derived hyperpolarizing factor & bradykinin etc.

When endothelial cells undergo inflammatory activation, an increase in expression of adhesion molecules such as selectins, vascular cell adhesion molecule -1 (VCAM-1) & intercellular adhesion molecule -1 (ICAM-1) promotes the adherence of the inflammatory cells monocytes, neutrophils, lymphocytes and macrophages and recruitment of additional cytokines growth factors and MMPs. The increased expression of these cell adhesion molecules are brought out by the release of cytokines like IL-1β, IL-6, 8, TNF-α etc during the inflammatory process.

Cytokines could also induce vascular cell growth and migration. It was shown that the TNF-α and IL-6 induce VGEF expression in cultured A431 human endothelial carcinoma and skeletal myoblast cell lines. TNF-α and IL-1 also mediate adhesion molecule expression on endothelial cells and hence play a role in the migration of polymorphonuclear neutrophils (PMNs), lymphocytes and macrophages into the periodontal tissue.

Interaction between vascular cell adhesion molecule-1(VCAM-1) on endothelial cells and α4 integrins on leukocytes is thought to mediate the selective recruitment of eosinophils and lymphocytes that occurs in allergic diseases. IL-4 is associated with allergic conditions. It selectively increases expression of VCAM- 1 on endothelial cells in vivo which suggests that it could be responsible for expression of VCAM-1 in allergic disease. Using a combination of immunofluorescence, flow cytometry and Northern analysis, the effect of TNF-α and IL-4 on VCAM-1 expression were compared.

Bevilacqua MI et al (1985)[34] suggested that both TNF-α and IL-1 have been shown to act on endothelial cells to increase the attachment of polymorphonuclear neutrophils and monocytes and thus help to recruit these cells into sites of inflammation. TNF-α can initiate apoptosis. It can stimulate apoptosis in many cell types by the recruitment of the DED containing protein caspase-8 to the receptor complex following association of TRADD & FADD to TNRF-1. This receptor recruitment results in autocatalytic activation of caspase-8.it then initiates a hierarchical series of caspase activation steps culminating in the activation of effector caspase like caspase-3. Cytokines also promote adhesion of immune cells to EC and cause an increase in vascular permeability.

M H Thornhill (1991)[35] stated that IL-1 and TNF-α alone are unselective in terms of leukocyte adhesion. TNF-α along with IL-4 or IFN-ɣ plays a key role in determining the recruitment of a lymphocyte-predominant infiltrate in immune mediated inflammation, and also in initiating the transition from acute to chronic inflammation.

Springer et al (1994)[36] suggested that many of the endothelial surface cell adhesion molecules, including E-selectin, VCAM- l and ICAM- l, are increased by inflammatory cytokines, such as TNF-α, IL-1.

M F Lademarco et al (1995)[37] proposed that when TNF-α and IL-4 were combined, there was a synergistic increase in VCAM-1 expression and a dramatic prolongation of the appearance of VCAM-1 on the cell surface. This synergy results from a combination of transcriptional activation by TNF-α and the stabilization of resulting transcripts by IL-4. IL-4 allows sub threshold concentrations of TNF-α (concentrations that would not normally activate expression of adhesion molecules on the endothelium) to selectively increase VCAM-1 expression and to prolong its appearance on the surface of endothelial cells.

Dinarello CA et al (2011)[27] showed that the TNF-α acts in the cell migration process at multiple levels, inducing the up-regulation of adhesion molecules and the production of chemotactic cytokines involved in cell migration to sites of inflammation.

Clauss et al (2018)[38] demonstrated that endogenously expressed TNF-α facilitates the ability of VEGF to induce vascular permeability.

EFFECT OF TNF-α ON BONE CELLS

The failure to encapsulate this inflammatory reaction within the gingival tissue results in expansion of the response to adjacent alveolar bone. Occurrence of bone loss in response to an inflammatory reaction depends on the presence of sufficient concentration of inflammatory mediators in gingival tissue to activate pathways leading to bone resorption.

Mundy et al (1991)[39] demonstrated that the TNF-α molecules stimulate bone resorption by inducing the proliferation and differentiation of osteoclast progenitors and activating formed osteoclasts indirectly. It is now well accepted that a large consortia of cytokines, cell-signalling molecules and matrix metalloproteinase are deregulated and intimately involved in the pathogenesis of periodontitis. The task ahead of us is to identify the various roles of these important biological mediators of inflammation and how to control them.

Assuma R et al (1998)[9] demonstrated that in an animal model, *P.gingivalis*-soaked silk ligatures applied to posterior mandibular teeth to induce experimental periodontitis showed significant inflammatory cell recruitment and osteoclast formation surrounding bone in the control primates. Thus, infection with *P.gingivalis* in these animals was associated with expansion of the inflammatory front to alveolar bone. In contrast, antagonists to cytokines TNF-α and IL-1 reduced the appearance of inflammatory cells in this region and the formation of bone resorbing osteoclasts. These findings suggested that inhibition of the inflammatory mediators can prevent the inflammatory front from reaching alveolar bone, and it was associated with a reduction in bone loss in this animal model.

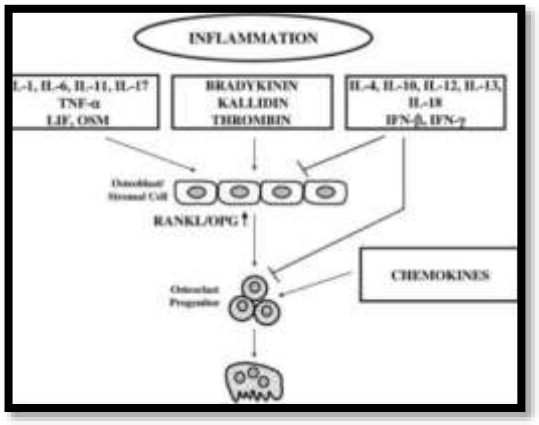


Fig 2: Stimulation and inhibition of osteoclast formation and bone resorption
(Courtesy: Journal of Periodontology, 2003[18])

Nakashima T et al (2000)[40] proposed that during an inflammatory response proinflammatory cytokines, such as IL-1β, 6, 11 and 17 and TNF-α, can induce osteoclastogenesis by increasing the expression of RANKL while decreasing OPG production in osteoblasts/stromal cells. Whereas anti-inflammatory mediators, such as IL-13 and IFN-ɣ, may lower RANKL expression and/or increase OPG expression to inhibit osteoclastogenesis.

Boyle WJ et al (2003)[41] suggested that principal feature of inflammatory-mediated bone loss in periodontitis is enhanced osteoclast activity without a corresponding increase in bone formation.

Graves DT et al (2003)[18] suggested that inflammation and bone loss are hallmarks of periodontal disease. They demonstrates that PD involves bacterially derived factors and antigens that stimulate a local inflammatory reaction leading to activation of the innate immune system. Proinflammatory molecules and cytokine networks play essential roles in the process of inflammatory reaction. IL-1 and TNF-α seem to be primary molecules that influence cells in the lesion. A cascade of events leads to osteoclastogenesis and bone loss via the receptor activator of nuclear factor-kappa B (RANK) - RANK ligand (RANKL) - osteoprotegerin (OPG) axis. The initial response to bacterial infection is a local inflammatory reaction resulting in activation of the innate immune system. Amplification of this initial localized response results in production and release of an array of cytokines and progression of inflammation through the gingival tissues.

Lerner UH et al (2016)[42] said that the inflammatory mediators must penetrate gingival tissue to reach within a critical distance to alveolar bone. Achieving critical concentrations of inflammatory mediators that lead to bone resorption depends on the

expression of proinflammatory cytokines, such as IL-1, 6, 11, and 17, TNF-α, leukemia inhibitory factor and oncostatin M. This is the opposite of the expression of anti-inflammatory cytokines and other mediators, such as IL-4, 10, 12, 13, and 18, as well as IFN-β and ɣ, which serve to inhibit bone resorption.

EFFECT OF TNF-α ON GINGIVAL FIBROBLASTS

The gingival fibroblast and periodontal ligament cells are involved in release of various cytokines which not only affect function of inflammatory cells but also remodelling of gingiva, periodontal ligament and alveolar bone. Another important role of gingival fibroblasts in PD pathogenesis is its long-lasting ability to produce inflammatory cytokines after LPS challenge.

In addition to LPS, HGFs also respond to outermembrane protein and polysaccharide of *P.gingivalis* by producing inflammatory cytokines. It was also found that primary HGFs and PDL fibroblasts respond to *P.gingivalis* by increasing gene expression for IL-1β, IL-6, IL-8, TNF-α and regulated on activation normal T-cell expressed and secreted (RANTES), with heterogeneity in responsiveness between fibroblasts from different donors and this may be important in determining susceptibility to periodontitis.

Clemens M J et al (1991)[20] found that the TNF-α is produced mainly by macrophages in response to agents such as lipopolysaccharide. IL-1 and TNF-α are key mediators of chronic inflammatory diseases and have the potential to initiate tissue destruction and bone loss in periodontal disease IL-1 has been shown to stimulate fibroblasts in culture to produce collagenase.

Agarwal et al (1995)[43], identified that cytokines induce the secretion of other cytokines. IL-1β induces the expression of IL-6, IL-8 and TNF-α in HGFs, and also acts in an autocrine manner to induce further IL-1β expression.

Yucel-Lindberg et al (1999)[44] found that IL-1β and TNF-α synergistically increase PGE2 production in HGFs and TNF-α was shown to upregulate PGE2 and COX production in HGFs via the JNK and NF-Kb signalling pathways.

Morton & Dongari-Bagtzoglou (2001)[45] found that IL-1β and TNF-a induce COX-2 in oral epithelial cells and IL-1β upregulates COX-2 expression in HGFs.

Uehara & Takada (2002)[46] said that during inflammation, resident GF are triggered by cytokines released by macrophages to enhance their synthesis of cytokines. Thus, IL-1β and TNF-α stimulate the expression and release of IL-6 and IL-11 in human gingival fibroblast. HGFs are responsive to LPS and constitutively express mRNA for a variety of TLRs and NLRs, stimulation of which leads to production of pro-inflammatory cytokines such as TNF-α, IL-6 and IL-8.

Palmqvist et al(2008)[31] conducted a study of cytokine expression in HGFs obtained from non-inflamed gingiva where there was a dose-dependent stimulation of IL-6 and LIF mRNA and protein by IL-1β and TNF-α and dose-dependent stimulation of IL-11 mRNA and protein by IL-1β.

In another study, TNF-α, IL-1β and PGF2α all stimulated IL-6 production in cultured HGFs and PGF-2α synergistically increased IL-6 production stimulated by TNF-α and IL-1β.[47] TNF-α also induced IL-1a and IL-1β production in HGFs, and this was synergistically enhanced by the presence of bradykinin. It was observed that the up sregulation of IL-6 production by HGFs that is induced by IL-1β is mediated by the p38

MAPK and NFkB signalling pathways. IL-1β and TNF-α act synergistically in stimulating IL-6 secretion by HGFs and this combination of cytokines was shown to be many hundreds of times more potent in stimulating IL-6 production than LPS.

Nakajima (2009)[47] reported increased serum levels of IL-1β, TNF-α, OC, soluble intercellular adhesion molecule (sICAM), IL-6, MMP-9 have been reported in experimental studies in animal models as well as in clinical studies in humans mediated by metalloproteinases.

Ceciki et al (2013)[48] reported that the TNF-α stimulates the production of chemokines involved in cell migration of HGFs to infected and inflamed sites, upregulates the production of IL-1β and IL-6 and is also correlated with extracellular matrix degradation and bone resorption through actions promoting the secretion of matrix metalloproteinases and RANKL.

SMOKING AND PERIODONTITIS

Bradford Hill (1965)[49] described 9 criteria for a factor to be considered as a causative/associated agent in "The environment and disease – association or causation." According to **Gelskey (1999)**[50] smoking meets majority of these criteria to varying degrees as follows:

Criterion	Definition	Example	Critique
Strength of association	The larger the relative risk (RR) or odds ratio (OR), the less likely the association is spurious	Hill cited a 200-fold increased mortality rate from scrotal cancer among chimney sweeps compared to workers not exposed to tar and mineral oils (71)	Even if an association appears weak, causality should not be ruled out since observed strength may be dependent on the relative prevalence of other variables (69)
Consistency	Repeated observation of association observed under varied study conditions	US Surgeon General's report on smoking and lung cancer cited 30 studies of various populations and study designs. All found a positive, statistically significant association (17)	Lack of consistency does not rule out causality because some effects are produced by the agent only under specific circumstances
Specificity	A cause is specific to an effect if the introduction of the exposure is followed by the effect and if removal results in resolution of the effect	Nifedipine-associated gingival overgrowth appears shortly after start of therapy and decreases upon withdrawal of the drug (72) suggesting specificity. However, specificity is not confirmed since overgrowth occurs in only 15% of those exposed to nifedipine	Specificity should be used as evidence for causality, but since it suggests single-factor causation, lack of evidence for specificity should not be used to refute cause
Temporality	A causal relationship requires that the exposure believed to cause a disease must precede the disease	Plaque allowed to accumulate for 21 days in periodontally healthy individuals induces gingivitis (73)	Temporal sequence is thought to be a *sine qua non* for causation; however, temporality is difficult to ensure in chronic diseases where onset is insidious, progress continuous, of long duration and modulated by the host (69)
Biologic gradient	A dose-response curve is observed when an increase or decrease in exposure corresponds to an increase or decrease in the frequency and/or severity of disease	A dose-response relationship was observed between the number of cigarettes smoked/day and lung cancer mortality among male British physicians, with the mortality rate being approximately 1 among nonsmokers compared to ~8 for 1–14 cigarettes/day, ~13 for 15–24 cigarettes/day, and ~25 for those smoking ≥25 cigarettes/day (65)	Not all associations that show a dose-response trend are causal. Confounding variables can produce the trend if the confounder itself shows a biologic gradient with the disease (69)
Biologic plausibility	The suspected cause should be biologically credible	A number of studies support the biologic credibility of a causal association between diabetes mellitus and periodontitis. Among those with diabetes, vascular changes occur in tissues of the periodontium; blood vessels thicken; degenerative vascular changes occur in gingival specimens, all credible explanations affecting delivery of nutrients/oxygen to the tissues (2, 74, 75)	Plausibility is based on current biological knowledge and therefore lack of biological credibility should not singularly nullify a causal hypothesis (9, 11)
Coherence	The association does not conflict with what is known about the natural history of the disease	The propensity for tobacco extracts to cause skin cancer in mice is coherent with the theory that use of tobacco causes lung cancer in humans (76)	Lack of coherence is dependent on current understanding of natural history of disease so that lack of coherence should not exclude an exposure as a potential cause
Analogy	A causal relationship becomes more believable if the exposure has been shown to have a causal effect on a related condition	That the causal role of thalidomide and rubella in birth defects has been demonstrated makes researchers ready to accept evidence that another drug used during pregnancy could do the same (11)	Caution should be exercised not to create analogies where they do not logically exist (69)
Experiment	The strongest support for causation is experimental evidence using randomized prospective studies	The etiologic role of bacterial plaque in gingivitis in human (73) and periodontitis using an animal model (77) has been confirmed	Beyond rare 'natural experiments' such as the observation that communities with naturally fluoridated water had experienced fewer dental caries than communities without fluoridated water (78), evidence for risk is seldom available from human studies

Fig 4: Bradford Hill's criteria for causation (Courtesy: Gelskey SC, 1999)

Studies suggest that the host bacterial interactions normally seen in chronic periodontitis are altered in smokers, resulting in more aggressive periodontal breakdown. This imbalance between bacterial challenge and host response may be due to changes in the composition of subgingival plaque, increase in the number and virulence of pathogenic organisms and changes in host response to bacterial challenge.

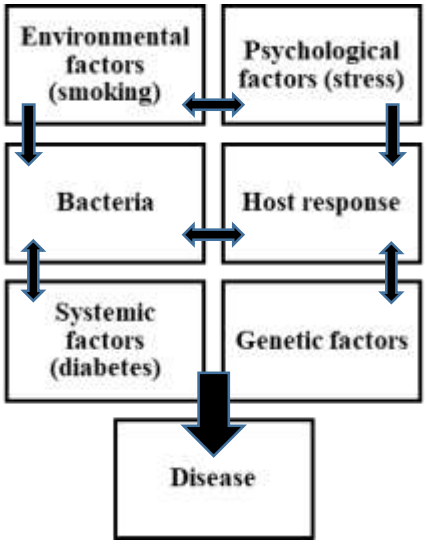


Fig 4: Model of interplay of etiologies, risk factors and risk indicators

ETIOLOGICAL FACTOR	IMPACT OF SMOKING
Microbiology	No effect on rate of plaque accumulation Increased colonization of periodontal pockets by pathogenic species
Immunology	Altered neutrophil chemotaxis, phagocytosis and oxidative burst Increased production of TNF-α and PGE2 Increased neutrophil collagenase and elastase
Physiology	Decreased gingival blood vessels and gingival bleeding Decreased GCF flow Decreased subgingival temperature

Fig 5: Impact of smoking on etiological factors of periodontitis

Preber and Bergstrom et al (1992)[51] reported that while smoking is accepted as a strong modifying factor for periodontal diseases, there is lack of consensus regarding its precise mechanisms. They reported that smoking does not influence the subgingival colonization of some important periodontal pathogens as smoking and non-smoking periodontitis patients largely exhibited the same microflora.

Haber (1994)[52] reported that clinically smokers commonly presented with fibrotic gingiva, with limited gingival redness and edema relative to disease severity; proportionally greater pocketing in anterior and maxillary lingual sites; gingival recession in anterior region; and a lack of association between periodontal status and oral hygiene of the patient.

Seow, Thong et al (1994)[53] examined the effects of nicotine on neutrophil function at concentrations simulating those found in oral tissues. The results demonstrated a dose-dependent suppression of chemotaxis and phagocytosis. They also examined the effects of nicotine on neutrophil function at concentrations found in oral tissues and showed an enhancement of neutrophil degranulation. As a result, the literature supports the fact that tobacco may contribute to the progression of periodontal disease, at least in part, through the release of proteases from periodontal neutrophils[58]

Martinez-Canut, Lorca et al (1995)[54] provided further evidence that smoking as a risk factor for chronic periodontitis is strengthened by the ability to demonstrate a dose-response (years of exposure) of tobacco products to the severity of periodontal disease. The literature evidence strongly supports the observation that the longer or the greater the number of cigarettes a patient smokes, greater will be the severity of periodontal disease.

Risk calculations by **Tomar and Asma (2000)**[55] suggests that 40% of chronic periodontitis cases may be attributed to smoking. Smokers are four times (odds ratio of 4.0) more likely to have chronic periodontitis than non-smokers. Smoking is associated with greater attachment loss, bone loss and tooth loss, but less signs of inflammation. Extensive clinical trials have also shown poorer response to non-surgical and surgical periodontal treatment in smokers.

Pauletto and Liede et al (2000)[56] explained that neutrophils are critical immune cells in the maintenance of periodontal health because of their multifaceted roles in the control of bacterial plaque. They contribute to the progression of periodontitis in chronic inflammatory responses. Smoking also affects multiple functions of neutrophils and can shift the net balance of neutrophil activities towards destruction. While tobacco smoke exposure increases the number of neutrophils found in the systemic circulation, the numbers of neutrophils entering the gingival sulcus and oral cavity remain unaffected or even reduced.

Van der Velden, Varoufaki et al (2003)[57] reported that smoking affects many aspects of the host's immune response, therefore it is probable that this may be its primary role in contribution to the pathogenesis of the disease.

Persson, Bergstrom et al (2003)[58] hypothesized that tobacco smoking increases the proteolytic activity of neutrophils. Though tobacco-induced release of proteolytic enzymes from neutrophils has not been demonstrated definitively in the periodontal tissues themselves, neutrophils have been considered to be a major source of the elastase and matrix metallo-proteases (MMPs) associated with periodontal disease destruction.

Palmer, Wilson et al (2005)[59] reported that neutrophil transmigration across the periodontal microvasculature is impeded in tobacco smokers.

EFFECT OF SMOKING ON PERIODONTAL TREATMENT

Preber and Bergstrom (1990)[60] observed the differences in clinical responses following non-surgical therapy in smokers versus non-smokers following non-surgical treatment.

Bolin et al (1993)[61] reported results from a 10-year radiographic follow-up study of alveolar bone loss which found that the progression of bone loss was significantly retarded in those who had quit smoking during the study compared with those who continued to smoke.

Tonetti et al (1995)[62] reported a significant difference in clinical attachment gains following guided tissue regeneration of intrabony defects for smokers and non-smokers of 2.1mm and 5.2mm respectively. They also reported that higher plaque levels were seen consistently in smokers compared with non-smokers which may also influence clinical outcomes.

Kaldahl, Johnson et al (1996)[63] conducted long term studies involving both surgical and non-surgical therapy show similar results, with non-smokers showing greater probing depth reduction and gain of attachment level.

Grossi, Zambon et al (1997)[64] reported greater probing depth reductions in non-smokers as compared to smokers and showed significantly greater pocket depth reductions (0.9-1.1mm) in non-smokers compared with smokers at 1 and 3 months following non-surgical therapy.

Papantonopoulos (1999)[65] showed that significantly more smokers (42.8%) than non-smokers (11.5%) required additional treatment at reevaluation, following nonsurgical therapy.

Bergstrom, Eliasson et al (2000)[66] reported that treatment for periodontal disease are likely to be more efficacious in non-smokers than in smokers, with the response of previous smokers being intermediate between these two groups.

Meinberg et al (2001)[67] in a radiographic study, reported significantly more bone loss at 12 months following non-surgical therapy in smokers compared with non-smokers.

Susin, Oppermann et al (2004)[68] reported that implementation of population-based smoking cessation programs may have a significant impact on the prevalence and progression of periodontal diseases.

Ramseier (2005)[69] stated that majority of studies investigating the effects of smoking cessation on periodontal disease acknowledge the benefits of smoking cessation counselling and concluded that smoking cessation may result in long-term benefits to the periodontium.

Preshaw et al (2005)[70] showed similar radiographic results over 12 months with former smokers as well as a significant reduction in probing depths and a higher incidence of probing depth reductions of 2 and 3 mm in the former smokers as compared to current smokers.

Georgios et al (2015)[71] concluded that active smokers could be candidates for periodontal procedures. However, the magnitude of the therapeutic effect could be compromised in smokers compared with non-smokers. Therefore, cigarette smokers

should be encouraged to quit smoking and thoroughly informed preoperatively of substantial reduction in clinical outcomes compared with non-smokers.

Shereef et al (2015)[72] proved a significant difference in CAL between smokers and nonsmokers. Given the evidence that smokers have the worse periodontal disease than nonsmokers, and the magnitude and predictability of clinical improvements after treatment are significantly reduced in smokers, smoking cessation counseling is considered important.

Leite et al (2018)[73] reported that smoking cessation reduced the risk for periodontitis onset and progression, and improved the outcomes of nonsurgical periodontal therapy. Tobacco smoking, therefore, is an important risk factor to be assessed for periodontitis.

Antonella et al (2018)[74] based on systematic review found that people who smoke will experience less reduction in probing depth than nonsmokers. However there is no evidence of s difference in gain in CAL between non-smokers and smokers or a reduction in bleeding on probing between smokers and non-smokers.

Masoome et al (2018)[75] concluded that smoking habit contributes to an unfavourable clinical response to nonsurgical and surgical antiinfectious therapies and to regenerative and plastic periodontal procedures. Scaling and root planning alone presents a modest effectiveness in improving clinical parameters and in reducing the levels of periodontal pathogens in smokers; therefore, adjunctive therapeutic approaches have been suggested to treat periodontitis in smokers.

SALIVA AS A DIAGNOSTIC MEDIUM

Saliva has been proposed as a non-invasive diagnostic fluid that could be used in the diagnosis of oral and systemic diseases. The levels of salivary biomarkers like cytokines, could potentially be used as a surrogate marker to distinguish periodontally healthy individuals from subjects with periodontitis.

The idea of using saliva in diagnostics was made in the second half of the 20th Century[76,77]. The earliest "sialochemical" studies on oral fluids were conducted by **Michaels and Kirk in the 1900's**, each of whom examined saliva for specific components that would be diagnostic for various systemic conditions, including gout and rheumatism[78].

The three most common approaches for collection of unstimulated saliva are draining, spitting, and suction methods[79,80,81,82]. The constituents of saliva vary depending on the harvesting method and the degree of salivary flow.

Whole saliva represents pooled samples with contribution from all periodontal sites and thus helps to provide an overall assessment of diseases status[83]. Saliva can be collected with or without stimulation. Stimulated saliva is collected by masticatory action (i.e., from a subject chewing on paraffin) or by gustatory stimulation (the subject's tongue; **Mandel, 1993**)[84]. Stimulation affects the quantity of saliva collected, the concentrations of some constituents and the pH of the fluid.

Unstimulated saliva is collected without exogenous gustatory, masticatory, or mechanical stimulation. Unstimulated whole saliva often correlates to systemic clinical conditions more accurately than stimulated saliva, since materials used to stimulate

flow may change salivary composition. Levels of biochemical and immunological components measured in saliva may reflect blood levels.

Navaszesh et al (1993)[85] suggested that the best two ways to collect whole saliva are the draining method, in which saliva is allowed to drip off the lower lip, and the spitting method, where the subject expectorates saliva into a test tube.

Substitution of blood samples with saliva in analysis of biomarkers is of considerable interest because collection of saliva is non-invasive and does not have any of the risks associated with collection of blood.

SALIVA AS A BIOMARKER

For the past two decades, salivary diagnostic approaches have been developed to monitor oral diseases such as periodontal diseases[81], to assess caries risk[82] and oral cancer[83]. Recently, due to the combination of emerging biotechnologies in the field of salivary diagnostics, a huge number of medically valuable analytes in saliva are gradually unveiled and some of them represent biomarkers for different diseases including cancer, infections, etc. The greatest challenge of salivary diagnostics lies in identifying disease diagnostic markers and successfully translating these research efforts from the laboratory into the clinic. Robust scientific platforms for saliva biomarker discovery have been developed to empower salivary diagnostics to become an approach for health surveillance[89].

Substitution of saliva samples for blood in analysis of biomarkers is of considerable interest because collection of saliva is less invasive and does not have any of the risks associated with collection of blood. While there is some information about single biomarkers in saliva (such as cortisol), the correspondence to blood levels varies

widely by biomarker and to date there is little evidence regarding how well blood levels of specific cytokines or other biomarkers are represented in saliva.

The correlation between clinical features of periodontal disease and salivary biomarkers has been evaluated for three aspects of periodontitis - inflammation, collagen degradation and bone turnover. The use of saliva as a periodontal diagnostic aid has been the subject of considerable research activity, and proposed markers for disease include proteins of host origin (i.e. cytokines, enzymes, immunoglobulins), phenotypic markers (epithelial keratins), host cells, hormones (cortisol), bacteria and bacterial products, volatile compounds and ions.

Zambon et al (1985)[90] proposed that the effectiveness of periodontal treatment might be monitored by changes in levels of specific bacterial enzymes in whole saliva.

Chiappin et al[91] said that Salivary glands have rich vasculature from which saliva is filtered and processed. The components of saliva may either originate entirely from the salivary glands or be derived from the blood by passive diffusion or active transport. In cases where components in saliva are derived from the blood, levels of biochemical and immunological components measured in saliva may reflect blood levels.

Frodge et al (2008)[92] evaluated salivary concentrations of TNF-α, RANKL, and ICTP in 35 subjects with moderate to severe chronic periodontitis in comparison with 39 healthy controls. The authors reported that salivary TNF-α levels were significantly elevated in chronic periodontitis patients suggesting that it could facilitate the screening, diagnosis and management of periodontal diseases.

Balwant Rai et al (2008)[93] estimated salivary TNF-α levels in periodontitis and healthy normal and reported significantly higher levels Salivary TNF-α in periodontitis patients. He concluded that saliva provides an ideal medium for the detection of proinflammatory markers of the oral cavity.

Gursoy et al (2009)[94] conducted a study to analyse the levels of IL-1, IL-6, & TNF-α in saliva of 84 chronic periodontitis patients and 81 controls. There was statistical difference in the level of IL-6 & TNF-α.

Sheeja et al (2015)[95] reported salivary TNF-α levels is significantly elevated in chronic periodontitis when compared to healthy subjects but similar results were not observed in GCF.

Ngamchuea et al (2018)[96] concluded that to date there has been little published regarding how saliva collection technique affects recovery of specific bio-markers. Whole saliva represents pooled samples with contribution from all periodontal sites and thus helps to provide an overall assessment of diseases status.

Lee et al (2018)[97] reported that saliva contains potential biomarkers for predicting the sensitivity and monitoring the response to non-surgical periodontal therapy.

MATERIALS AND METHODS

STUDY PROTOCOL

- Institutional Ethical Committee approval obtained
- Medical History and Informed Consent obtained
- Periodontal Examination using clinical parameters namely, Gingival Bleeding Index, Plaque Index, Probing Pocket Depth and Clinical Attachment Level.
- Collection of saliva samples
- Phase-I therapy was given to Group I and Group II patients.
- Quantification of TNF-α levels in saliva of all subjects before and after phase-I therapy by ELISA

Patients who attended the outpatient Department of Periodontology, Tamilnadu Government Dental College and Hospital, Chennai were randomly selected and enrolled in the study. The purpose of the study was explained to all the subjects (ANNEXURE I & II) and an informed consent was obtained (ANNEXURE III & IV). The patients were informed that this research work was in no way directly related to the therapy or cure of the disease. The study was undertaken following approval from the Institutional Review Board.

SAMPLE CRITERIA

A total of 30 subjects was selected. The subjects wire divided into two groups based on the habit of smoking:

- Group 1 – Control group (15 subjects)
- Group 2 – Study group (15 subjects)

Both the groups were further divided into baseline and 3months postoperative groups:

- Group 1 – Control group
    - 1A – Non-smokers with generalized chronic periodontitis before phase-I periodontal therapy.
    - 1B – Non-smokers with generalized chronic periodontitis after phase-I periodontal therapy.
- Group 2 – Study group
    - 2A – Smokers with chronic periodontitis before phase-I periodontal therapy.
    - 2B – Smokers with chronic periodontitis after phase-I periodontal therapy.

ELIGIBILITY CRITERIA

- **Inclusion criteria:**
    - Age 30-60years.
    - Gender – male.
    - Systemically healthy individuals.
    - Generalized Chronic Periodontitis - loss of attachment ≥5mm and bone loss in radiograph (involving more than 30% of the sites).
    - Current smokers - ≥10 cigarettes per day for more than 1 year (only cigarette smokers).
    - Willingness to participate in study program.

- **Exclusion criteria:**
  - Patients with underlying systemic diseases.
  - History of periodontal treatment done in last 6 months.
  - Patients with other oral habits like tobacco and pan chewing.
  - Patients who received medications like antibiotics, anti-inflammatory, steroids within past 4 to 6 months.
  - Patients under bisphosphonates medication.
  - Alcoholics.

DATA COLLECTION

- **Armamentarium:**
  - **For Clinical Examination:**
    - Mouth mirror
    - Williams periodontal probe
    - Kidney tray
    - Cotton roll
    - Sterilized disposable gloves, head cap, facemask
  - **For Phase I therapy:**
    - Mouth mirror
    - Williams probe
    - Kidney tray
    - Cotton rolls
    - Sterilized disposable gloves, head cap, facemask
    - Disposable syringes

- Local anaesthetic solution (Lignocaine)
- Hu-Friedy Gracey Currettes

- **For saliva sample collection**
  - Disposable syringe
  - Eppendorf's tube

➢ **<u>Clinical parameters:</u>**

Using Proforma (Annexure V), case history was taken and the following clinical parameters were estimated in the subjects of Group-I and Group-II before and after phase-I therapy.

- Plaque index – ***Silness and Loe 1964***[98]
- Gingival bleeding index – ***Ainamo and Bay 1975***[99]
- Probing depth in mm (PD) – ***Carranza 10th ed***[100]
- Clinical attachment level in mm (CAL) – ***Carranza 10th ed***[100]

a) Plaque Index *(Silness and Loe 1964)*[98]

- All teeth were examined at 4 sites each (disto-facial, facial, mesio-facial, lingual / palatal) and were scored as follows:
- **Scoring Criteria:**
  - **Score 0:** No plaque in the gingival area.
  - **Score 1:** A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque is recognized only by running a probe across the tooth surface.

- **Score 2:** Moderate accumulation of plaque within the gingival pocket and on the gingival margin and/or adjacent tooth surface that can be seen by the naked eye.
- **Score 3:** Abundance of soft deposits within the gingival pocket and/or on the gingival margin and adjacent tooth surface.

- **Calculation**:
  - Plaque index per tooth = Total score/4
  - Plaque index per individual $= \frac{Total\ PI\ per\ tooth}{Total\ number\ of\ teeth\ examined}$
  - **Interpretation:**
    - Score 0 – Excellent oral hygiene
    - 0.1 to 0.9 – Good oral hygiene
    - 1.0 to 1.9 – Fair oral hygiene
    - 2.0 to 3.0 - Poor oral hygiene

b) Gingival Bleeding Index *(Ainamo & Bay 1975)*[99] (%)

- Starting distobuccally, the probe was gently inserted into the sulcus and run to the buccal and mesial surfaces of every tooth at an angle of about 45º. This was repeated for all the teeth present. Similarly probing was carried out at palatal/lingual sites. The total number of bleeding sites per tooth was thus recorded for every tooth except the third molar.
- **Scoring Criteria:**
  - Positive score - Presence of bleeding within 10 seconds
  - Negative score - Absence of bleeding
  - % of bleeding sites = $\frac{Total\ number\ of\ positive\ score\ X\ 100}{Total\ number\ of\ surfaces\ of\ all\ teeth}$

c) Probing Pocket Depth (PPD) (mm)

- Probing Pocket Depths were measured from the gingival margin to the base of the pocket in millimeters using William's Periodontal Probe.
- The probe was walked within the gingival sulcus along the circumference of the tooth. Keeping the probe parallel to the long axis of the selected tooth, six measurements were made per tooth (Mesiobuccal, Distobuccal, Midbuccal, Mesiolingual, Distolingual and Midlingual).

d) Clinical Attachment Level (CAL) (mm)

- Clinical Attachment Level was measured from the Cemento–Enamel Junction (CEJ) to the base of the pocket using William's Periodontal Probe.
- When the gingival margin was located on the anatomic crown, the level of the attachment was determined by subtracting from the probing depth, the distance from the gingival margin to the CEJ. If both were the same, the loss of attachment was calculated to be zero.
- When the gingival margin coincided with the CEJ, the loss of attachment was calculated as equalling the probing depth.
- When the gingival margin was located apical to the CEJ, the loss of attachment was greater than the probing depth and therefore the distance between the CEJ and the gingival margin were added to the PD.
- Three measurements were made on the buccal aspect and three on the lingual aspect of each tooth – total of six sites per tooth (Mesiobuccal, Midbuccal, Distobuccal, Mesiolingual, Midlingual, and Distolingual).

- **Radiographic parameters**

  - Radiographs were taken to assess bone loss

- **Routine blood investigations**

  - Haemoglobin %
  - Bleeding time and clotting time
  - Total leukocyte count
  - Differential leukocyte count

- **Saliva sample collection**

Saliva sample was collected from the subjects before taking breakfast after rinsing mouth with water. Following this subjects were instructed to spit the pooled saliva (2ml) from the floor of the mouth into Eppendorf's tube. Saliva samples were taken for all subjects (both group I and II) at base line before therapy and 3 months postoperatively after therapy. The samples were centrifuged at 2600rpm and stored at -80° C and used for further analysis by ELISA.

- **Estimation of salivary TNF-α**

Quantitative determination of salivary TNF-α was done by Enzyme-Linked Immunosorbent Assay (ELISA) method (Bioassay Technology Laboratory®).

- **Assay principle**
    - TNF-α was added to the wells pre-coated with TNF-α monoclonal antibody.
    - Incubation buffer was added and incubated for 2 hours at room temperature.
    - A biotin-conjugated anti-Human TNF-α antibody was added which binds to Human TNF-α.
    - TNF-α antibody was washed away during the washing step. Streptavidin-HRP was added which binds to the biotin-conjugated anti-Human TNF-α antibody.
    - After incubation for 1 hour at room temperature unbound Streptavidin-HRP was washed away during the next washing step.
    - Substrate solution was then added and color developed in proportion to the amount of Human TNF-α.
    - The reaction was terminated by addition of acidic stop solution and absorbance was measured at 450 nm.

- **Reagents used**

Components	**Quantity**
Standard Solution (960ng/L)	0.5ml x1
Pre-coated ELISA Plate	12 * 8 well strips x1
Standard Diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop Solution	6ml x1
Substrate Solution A	6ml x1
Substrate Solution B	6ml x1
Wash Buffer Concentrate (30x)	20ml x1
Biotin-Conjugate Anti-Human TNF-α Antibody	1ml x1

- **Reagent preparation**
    - All reagents were brought to room temperature before use.
    - Standard - It was strongly recommended that all standards and samples be run in duplicate. If the standard hasn't been run out, the remaining must be kept at -20°C. Diluted standard can't be reused. Dilution of standard solutions suggested used are as follows:

480ng/L	Standard No.5	120µl Original Standard + 120µl Standard diluent
240ng/L	Standard No.4	120µl Standard No.5 + 120µl Standard diluent
120ng/L	Standard No.3	120µl Standard No.4 + 120µl Standard diluent
60ng/L	Standard No.2	120µl Standard No.3 + 120µl Standard diluent
30ng/L	Standard No.1	120µl Standard No.2 + 120µl Standard diluent

Standard	S5	S4	S3	S2	S1
960ng/L	480ng/L	240ng/L	120ng/L	60ng/L	30ng/L

    - Wash Buffer - 20ml of Wash Buffer Concentrate was diluted 30x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals were formed in the concentrate, it was mixed gently until the crystals completely dissolved.

- **Assay Procedure**
    - All reagents, standard solutions and samples were prepared as per manufacturer instructions. All reagents were brought to room

temperature before use and the assay was performed at room temperature.

- The number of strips required for the assay were determined. The strips were inserted in the frames for use. The unused strips were stored at 2-8°C.
- 50µl standard was added to standard well. 40µl sample was added to sample wells and followed by that 10µl anti-TNF-α antibody was added to sample wells. Then 50µl streptavidin-HRP was added to sample wells and standard wells and mixed well.
- The plate was covered with a sealer and incubated for 60 minutes at 37°C.
- The sealer was removed and the plate was washed 5 times with wash buffer. Wells were soaked with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, all wells were aspirated and washed 5 times with wash buffer, overfilling wells with wash buffer. The plate was blotted onto paper towels or other absorbent material.
- 50µl substrate solution A was added to each well and then 50µl substrate solution B was added to each well. Plate was covered with a new sealer and incubated for 10 minutes at 37°C in the dark.
- 50µl Stop Solution was added to each well, the blue color changed into yellow immediately.
- The optical density (OD value) of each well was determined immediately using a microplate reader set to 450 nm within 30 min after adding the stop solution.

- **Summary**

All reagents, samples and standards prepared

↓

Samples, standards and ELISA solutions added and Incubated for 1 hour minutes at 37°C.

↓

The plate washed 5 times.

↓

Substrate solution A and B added and Incubated for 10 minutes at 37°C for color development.

↓

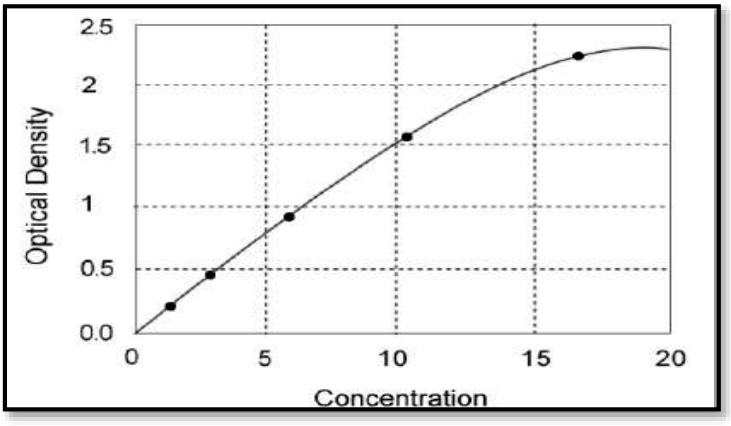
Stop solution added.

↓

The OD value were read within 10 minutes and calculations were done.

- **Calculations**

A standard curve was constructed by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and a best fit curve was drawn through the points on the graph. These calculations were performed with computer-based curve-fitting software and the best fit line was determined by regression analysis. If the standard have been diluted, the concentration read from the standard curve were multiplied by the dilution factor.



PHOTOGRAPH 1: GROUP-I (CONTROL GROUP)

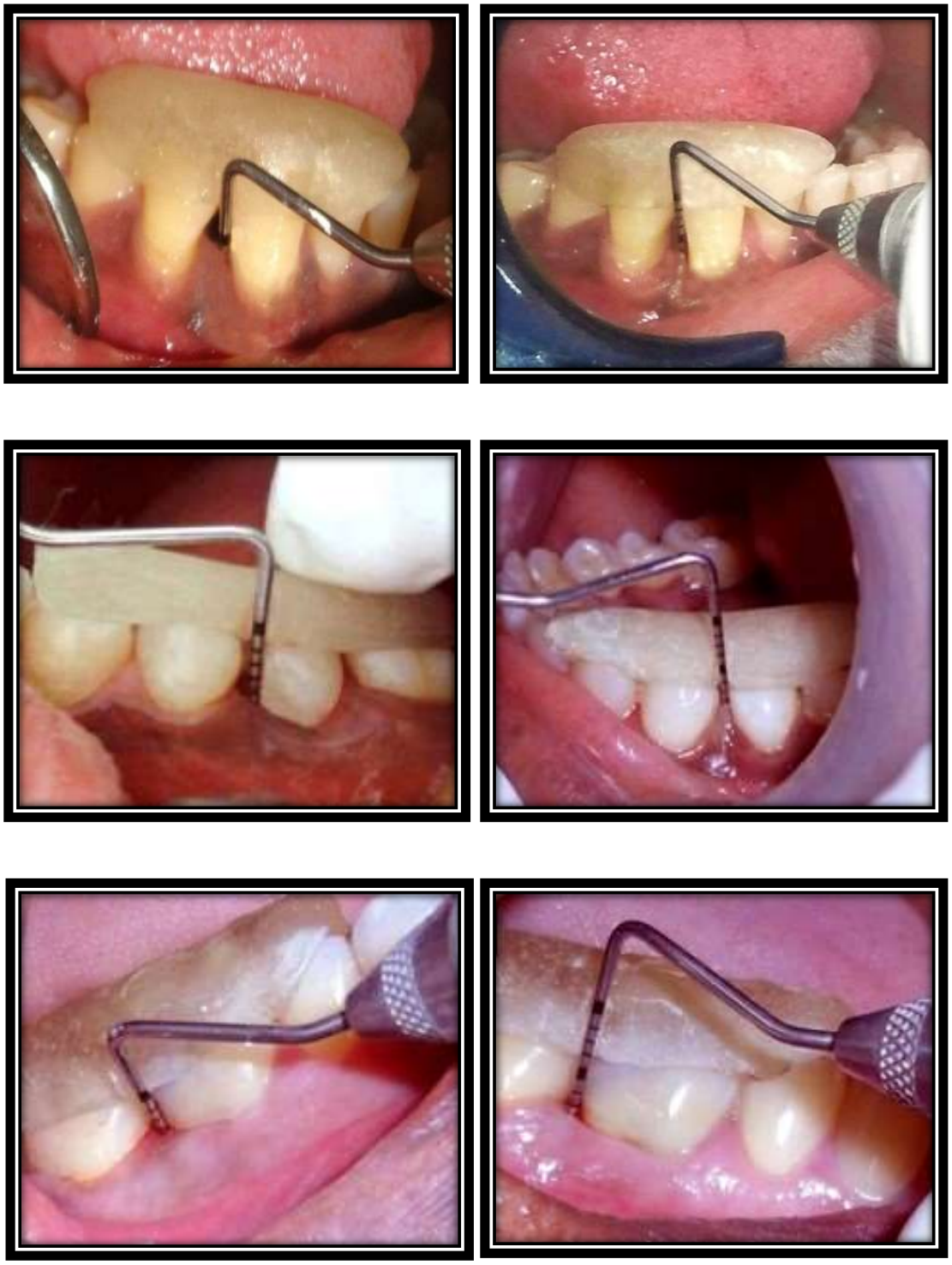
PHOTOGRAPH 2: GROUP-II (STUDY GROUP)

PHOTOGRAPH 3: ARMAMENTARIUM FOR CLINICAL EXAMINATION

PHOTOGRAPH 4: ARMAMENTARIUM FOR PHASE-I THERAPY

PHOTOGRAPH 5: EPPENDORF'S TUBE WITH SALIVA SAMPLE

PHOTOGRAPH 6: PROBING POCKET DEPTH MEASUREMENT BEFORE AND AFTER PHASE-I THERAPY

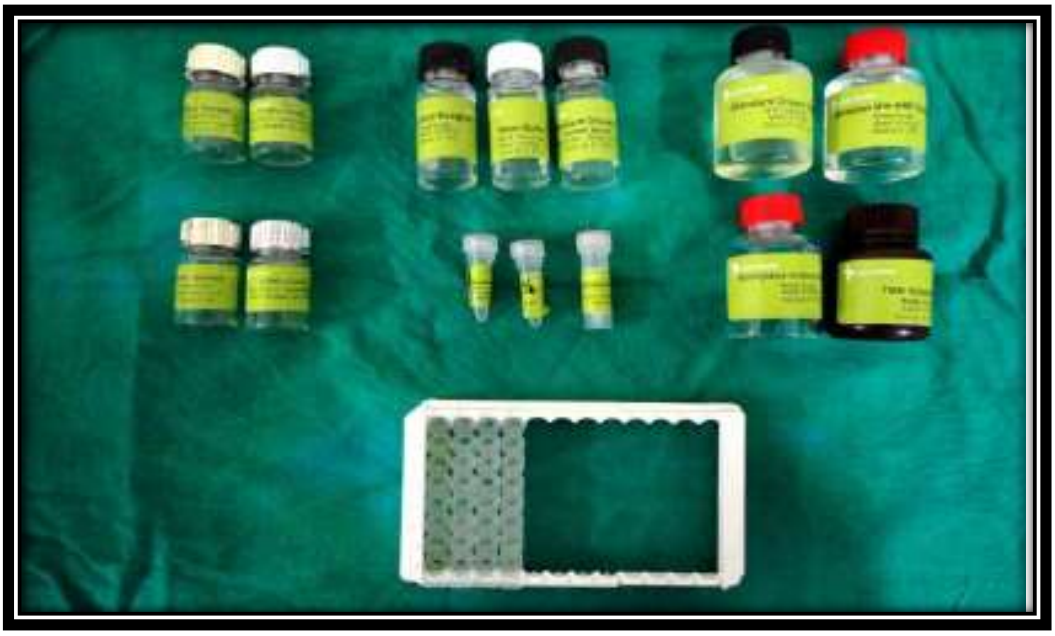


PHOTOGRAPH 7: REFRIGERATOR FOR SAMPLE STORAGE

PHOTOGRAPH 8: COOLING CENTRIFUGE

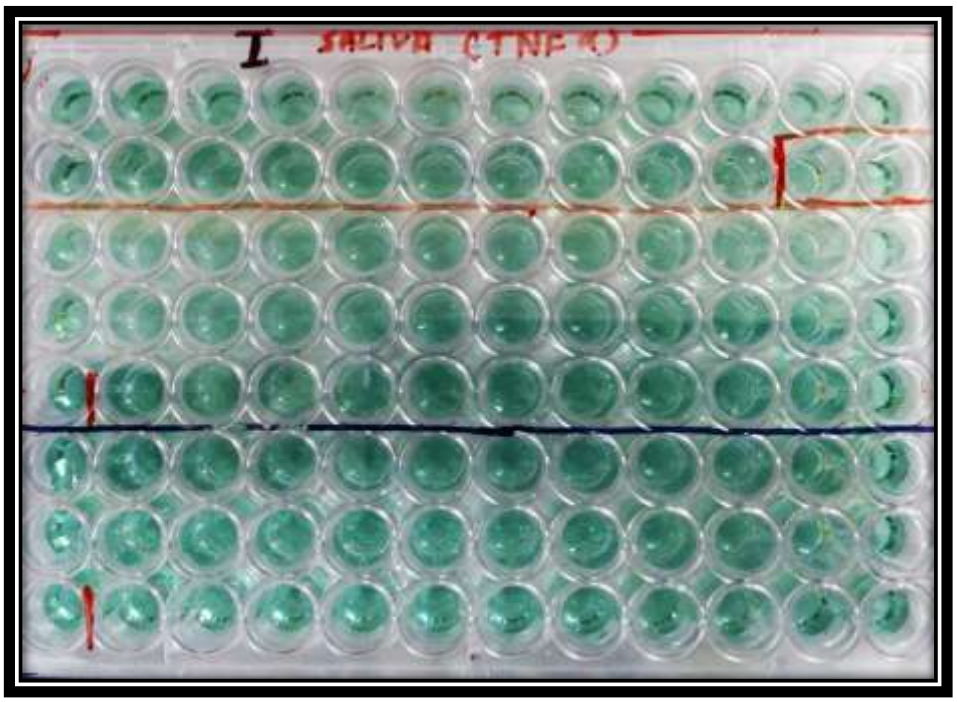
PHOTOGRAPH 9: ARMAMENTARIUM USED FOR SAMPLE PREPARATION

PHOTOGRAPH 10: REAGENTS USED



PHOTOGRAPH 11: BIO-RAD ELISA READER

PHOTOGRAPH 12: ELISA MICROPLATE



STATISTICAL ANALYSIS

The statistical package SPSS V20 (Statistical Package for Social Science, Version 120; SPSS Inc; Chicago IL, USA)) was used for statistical analysis.

a. Paired T test was used for intragroup comparison of clinical parameters and salivary TNF-α level at baseline and 3 months post-op.
b. Unpaired T test was used for intergroup comparison of clinical parameters and salivary TNF-α level at baseline and 3 months post-op.
c. Correlation coefficient between clinical parameters and salivary TNF-α level at baseline and 3 months post-op was obtained using Pearson Correlation Test.

- **Paired T test**

  For a comparison of more than one pair of split sample test results, the t-test for paired measurements can be used. Typically, this test uses the difference between each of the paired tests and determines whether the result is statistically different from 0, which is the expected theoretical result when testing separate portions of a single product. Thus it is the difference within pairs, not between pairs, that is being tested. The form of the t-test for paired measurements is:

$$t = \frac{\left|\overline{X}_d\right| - \left|X_O\right|}{\frac{s_d}{\sqrt{n}}}$$

Where,

$\overline{X_d}$ = Average of the differences between the individual split sample test results

X_O = The value of the expected difference between split sample tests. In most cases X0=0, as there is no expected difference between contractor and agency tests. However, X0 could reflect an expected correlation value between testing entities.

S_d = Standard deviation of the differences between the split sample test results.

n = Number of split samples

- **Unpaired T test (Independent T test)**

The independent T-test is used to compare the statistical significance of a possible difference between the means of two groups on some independent variable and the two groups are independent of one another. The formula for the independent T-test is:

$$t = \frac{X_1 - X_2}{\sqrt{\left(\frac{SS_1 + SS_2}{n_1 + n_2 - 2}\right)\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

Where,

X_1 = is the mean for group 1

X_2 = is the mean for Group 2

SS_1 = is the sum of squares for group 1

SS_2 = is the sum of squares for group 2

n_1 = is the number of subjects in group 1

n_2 = is the number of subjects in group 2.

The sum of squares is a new way of looking at variance. It gives us an indication of how spread out the scores in a sample are. The t-value is the difference between the two means divided by their sum of squares and taking the degrees of freedom into consideration.

$$SS_1 = \sum X_1^2 - \frac{\left(\sum X_1\right)^2}{n_1}$$

The p value or calculated probability is the estimated probability of rejecting the null hypothesis (H0) of a study question when that hypothesis is true. The

smaller the p value, the more significant the result is said to be. All P-values are two tailed, and confidence intervals were calculated at the 95% level. Differences between the two populations were considered significant when $p < 0.05$.

- **Pearson correlation**

Pearson correlation (Bivariate) was used to analyze the strength of association between the investigated variables. The correlation coefficient (r) was interpreted as follows:

0.0 - 0.1 - Trivial

0.1 - 0.3 - Low

0.3 - 0.5 - Moderate

0.5 - 0.7 - High

0.7 - 0.9 - Very high

0.9 - 1 - Nearly perfect

RESULTS

GINGIVAL BLEEDING INDEX (%)

- **Intragroup comparison**

  - **Group-I**: The mean gingival bleeding index score at baseline was 86.671±1.997 and at 3 months post-op was 20.829±1.284. The mean difference in gingival bleeding index score from baseline to 3 months post-op was 67.168±0.715 which was found to be statistically significant (p=0.000).

  - **Group-II**: The mean gingival bleeding index score at baseline was 83.521±1.239 and at 3 months post-op was 19.503±1.652. The mean difference in gingival bleeding index score from baseline to 3 months post-op was 62.692±1.411 which was also found to be statistically significant (p=0.000).

- **Intergroup comparison**

  - The mean difference between group I and group II at baseline was 3.150±2.350 which was statistically not significant (p=0.095) and at 3 months post-op was 1.326±2.092 which was also statistically not significant (p=0.265).

PROBING POCKET DEPTH (mm)

- **Intragroup comparison**

  - **Group-I**: The mean probing pocket depth at baseline was 5.552±0.283 and at 3 months post-op was 3.186±0.189. The mean difference in probing pocket depth from baseline to 3 months post-op was 2.366±0.829 which was found to be statistically significant (p=0.000).

  - **Group-II**: The mean probing pocket depth at baseline was 6.355±0.267 and at 3 months post-op was 3.589±0.180. The mean difference probing pocket depth from baseline to 3 months post-op was 2.766±0.123 which was also found to be statistically significant (p=0.000).

- **Intergroup comparison**

  - The mean difference between group I and group II at baseline was 0.803±0.389 which was statistically significant (p=0.024) and at 3 months post-op was 0.403±0.262 which was statistically not significant (p=0.067).

CLINICAL ATTACHMENT LEVEL (mm)

- **Intragroup comparison**

  - **Group-I**: The mean clinical attachment level at baseline was 5.604±0.283 and at 3 months post-op was 3.238±0.192. The mean difference in clinical attachment level from baseline to 3 months post-op was 2.366±0.825 which was found to be statistically significant (p=0.000).

  - **Group-II**: The mean clinical attachment level at baseline was 6.406±0.268 and at 3 months post-op was 3.641±0.182. The mean difference in clinical attachment level from baseline to 3 months post-op was 2.765±0.122 which was also found to be statistically significant (p=0.000).

- **Intergroup comparison**

  - The mean difference between group I and group II at baseline was 0.802±0.390 which was statistically significant (p=0.024) and at 3 months post-op was 0.419±0.288 which was also statistically significant (p=0.000).

PLAQUE INDEX

- **Intragroup comparison**

  - **Group-I**: The mean plaque index score at baseline was 2.214±0.103 and at 3 months post-op was 0.874±0.056. The mean difference in plaque index score from baseline to 3 months post-op was 1.340±0.075 which was found to be statistically significant (p=0.000).

  - **Group-II**: The mean plaque index score at baseline was 2.198±0.099 and at 3 months post-op was 0.895±0.052. The mean difference in plaque index score from baseline to 3 months post-op was 1.302±0.062 which was also found to be statistically significant (p=0.000).

- **Intergroup comparison**

  - The mean difference between group I and group II at baseline was 0.016±0.143 which was statistically not significant (p=0.454) and at 3 months post-op was 0.20±0.077 which was also statistically not significant (p=0.395).

SALIVARY TNF-α LEVEL

- **Intragroup comparison**

  - **Group I**: The mean salivary TNF-α level at baseline was 44.429±0.817 and at 3 months post-op was 28.881±0.533. The mean reduction in salivary TNF-α level from baseline to 3 months post-op was 15.548±0.284 which was found to be statistically significant (p=0.000).

  - **Group II**: The mean salivary TNF-α level at baseline was 54.160±1.049 and at 3 months post-op was 38.995±0.756. The mean reduction in salivary TNF-α level from baseline to 3 months post-op was 15.164±0.293 which was also statistically significant (p=0.000).

- **Intergroup comparison**

  - The mean difference between group I and group II at baseline was 9.730±1.330 which was found to be statistically significant (p=0.000) and at 3 months post-op was 10.114±0.925 which was statistically non-significant (p=0.000).

COMPARISON OF GINGIVAL BLEEDING INDEX

TABLE 1

GROUP I (CONTROL GROUP)

S.NO	AGE	SEX	GBI (%)	
			BL	3M
1.	35	M	75.01	10.71
2.	51	M	97.32	29.46
3.	40	M	86.32	18.75
4.	31	M	75.40	14.28
5.	32	M	91.07	19.64
6.	51	M	92.85	26.54
7.	43	M	84.82	15.17
8.	31	M	96.42	28.57
9.	32	M	91.96	24.10
10.	60	M	90.17	20.53
11.	33	M	76.78	12.50
12.	41	M	93.75	27.67
13.	40	M	86.60	16.96
14.	49	M	75.89	11.60
15.	41	M	85.71	16.07

TABLE 2

GROUP II (STUDY GROUP)

S.NO	AGE	SEX	GBI (%)	
			BL	3M
1.	47	M	85.71	26.78
2.	37	M	84.82	25.01
3.	46	M	89.28	25.89
4.	45	M	71.31	13.39
5.	43	M	84.84	14.63
6.	39	M	82.14	12.19
7.	46	M	83.92	21.42
8.	39	M	80.48	23.21
9.	38	M	83.03	20.49
10.	41	M	90.17	15.17
11.	40	M	79.46	20.53
12.	35	M	80.35	19.64
13.	44	M	89.46	22.32
14.	36	M	81.25	24.10
15.	40	M	86.60	27.67

COMPARISON OF PROBING POCKET DEPTH

TABLE 3

GROUP I (CONTROL GROUP)

S.NO	AGE	SEX	PPD (mm)	
			BL	3M
1.	35	M	5.68	3.96
2.	51	M	4.89	2.36
3.	40	M	5.86	3.88
4.	31	M	7.52	3.92
5.	32	M	4.21	2.12
6.	51	M	5.01	3.13
7.	43	M	6.33	4.12
8.	31	M	6.71	2.18
9.	32	M	4.63	2.49
10.	60	M	5.34	3.26
11.	33	M	7.15	4.05
12.	41	M	5.13	3.36
13.	40	M	4.24	2.68
14.	49	M	6.49	3.74
15.	41	M	4.09	2.54

TABLE 4

GROUP II (STUDY GROUP)

S.NO	AGE	SEX	PPD (mm)	
			BL	3M
1.	47	M	7.81	4.38
2.	37	M	6.74	3.38
3.	46	M	5.69	3.61
4.	45	M	6.04	3.36
5.	43	M	4.63	2.49
6.	39	M	7.39	4.32
7.	46	M	6.88	4.10
8.	39	M	7.13	3.96
9.	38	M	4.54	2.07
10.	41	M	7.42	4.59
11.	40	M	5.43	3.19
12.	35	M	7.39	4.11
13.	44	M	5.62	3.10
14.	36	M	6.76	3.49
15.	40	M	5.86	3.69

COMPARISON OF CLINICAL ATTACHMENT LEVEL

TABLE 5

GROUP I (CONTROL GROUP)

S.NO	AGE	SEX	CAL (mm)	
			BL	3M
1.	35	M	5.71	4.01
2.	51	M	4.91	2.42
3.	40	M	5.91	3.92
4.	31	M	7.58	3.98
5.	32	M	4.23	2.16
6.	51	M	5.09	3.18
7.	43	M	6.38	4.19
8.	31	M	6.78	2.22
9.	32	M	4.69	2.51
10.	60	M	5.39	3.31
11.	33	M	7.20	4.15
12.	41	M	5.19	3.41
13.	40	M	4.29	2.71
14.	49	M	6.54	3.81
15.	41	M	4.18	2.59

TABLE 6

GROUP II (STUDY GROUP)

S.NO	AGE	SEX	CAL (mm)	
			BL	3M
1.	47	M	7.85	4.45
2.	37	M	6.79	3.44
3.	46	M	5.72	3.66
4.	45	M	6.09	3.40
5.	43	M	4.68	2.53
6.	39	M	7.48	4.42
7.	46	M	6.93	4.15
8.	39	M	7.19	4.01
9.	38	M	4.59	2.12
10.	41	M	7.46	4.64
11.	40	M	5.48	3.22
12.	35	M	7.47	4.17
13.	44	M	5.66	3.15
14.	36	M	6.80	3.54
15.	40	M	5.91	3.72

COMPARISON OF PLAQUE INDEX

TABLE 7

GROUP I (CONTROL GROUP)

S.NO	AGE	SEX	PI	
			BL	3M
1.	35	M	2.67	0.88
2.	51	M	1.78	0.59
3.	40	M	1.86	0.64
4.	31	M	2.54	0.79
5.	32	M	1.71	0.56
6.	51	M	1.99	0.81
7.	43	M	2.88	0.99
8.	31	M	2.06	0.96
9.	32	M	1.82	0.61
10.	60	M	1.85	0.78
11.	33	M	2.26	0.89
12.	41	M	2.12	1.09
13.	40	M	2.31	1.14
14.	49	M	2.91	1.18
15.	41	M	2.46	1.21

TABLE 8

GROUP II (STUDY GROUP)

S.NO	AGE	SEX	PI	
			BL	3M
1.	47	M	2.09	0.93
2.	37	M	1.73	0.59
3.	46	M	2.34	1.06
4.	45	M	2.39	1.19
5.	43	M	1.89	0.66
6.	39	M	2.18	0.85
7.	46	M	2.42	1.15
8.	39	M	1.86	0.63
9.	38	M	2.97	1.22
10.	41	M	1.76	0.72
11.	40	M	2.83	0.97
12.	35	M	2.16	0.84
13.	44	M	1.84	0.76
14.	36	M	2.59	1.02
15.	40	M	1.92	0.84

COMPARISON OF SALIVARY TNF-α LEVEL

TABLE 9

GROUP I (CONTROL GROUP)

S.NO	AGE	SEX	SALIVARY TNF-α (pg/ml)	
			BL	3M
1.	35	M	45.09	29.30
2.	51	M	42.88	27.87
3.	40	M	45.77	29.75
4.	31	M	49.28	32.03
5.	32	M	39.89	25.92
6.	51	M	43.12	28.02
7.	43	M	46.63	30.39
8.	31	M	47.85	31.12
9.	32	M	41.62	27.05
10.	60	M	44.23	28.74
11.	33	M	48.81	31.72
12.	41	M	43.91	28.54
13.	40	M	40.54	26.35
14.	49	M	47.21	30.68
15.	41	M	39.61	25.74

TABLE 10

GROUP II (STUDY GROUP)

S.NO	AGE	SEX	SALIVARY TNF-α (pg/ml)	
			BL	3M
1.	47	M	59.92	43.14
2.	37	M	53.96	38.85
3.	46	M	51.54	37.10
4.	45	M	53.89	38.80
5.	43	M	48.53	34.94
6.	39	M	58.78	42.32
7.	46	M	56.32	40.55
8.	39	M	57.18	41.16
9.	38	M	48.39	34.84
10.	41	M	59.39	42.76
11.	40	M	49.10	35.36
12.	35	M	58.34	42.04
13.	44	M	49.91	35.93
14.	36	M	54.52	39.25
15.	40	M	52.63	37.89

DESCRIPTIVE STATISTICS OF GROUP-I AND GROUP-II

TABLE 11

GROUP-I

Variables	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
GBI BL	15	75.01	97.32	86.671	1.997	7.736
GBI 3M	15	10.71	29.46	19.503	1.652	6.400
PPD BL	15	4.09	7.52	5.552	.283	1.096
PPD 3M	15	2.12	4.12	3.186	.189	.735
CAL BL	15	4.18	7.58	5.604	.283	1.097
CAL 3M	15	2.16	4.19	3.238	.192	.746
PI BL	15	1.71	2.91	2.214	.103	.401
PI 3M	15	.56	1.21	.874	.056	.218
TNF-α BL	15	39.61	49.28	44.429	.817	3.167
TNF-α 3M	15	25.74	32.03	28.881	.533	2.065

TABLE 12

GROUP-II

Variables	N	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
GBI BL	15	71.31	90.17	83.521	1.239	4.801
GBI 3M	15	12.19	27.67	20.829	1.284	4.973
PPD BL	15	4.54	7.81	6.355	.267	1.036
PPD 3M	15	2.07	4.59	3.589	.180	.700
CAL BL	15	4.59	7.85	6.406	.268	1.041
CAL 3M	15	2.12	4.64	3.641	.182	.707
PI BL	15	1.73	2.97	2.198	.099	.386
PI 3M	15	.59	1.22	.895	.052	.204
TNF-α BL	15	48.39	59.92	54.160	1.049	4.064
TNF-α 3M	15	34.84	43.14	38.995	.756	2.928

COMPARISON OF CLINICAL PARAMETERS AT BASELINE AND 3MONTHS AFTER PHASE-I THERAPY

PAIRED T TEST

TABLE 13

GROUP –I

Variables	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval				
				Lower	Upper			
GBI BL - GBI 3M	67.168	2.772	.715	65.632	68.703	93.840	14	.000
PPD BL - PPD 3M	2.366	.829	.214	1.906	2.825	11.051	14	.000
CAL BL - CAL 3M	2.366	.825	.213	1.909	2.823	11.102	14	.000
PI BL - PI 3M	1.340	.293	.075	1.177	1.502	17.688	14	.000
TNF-α BL - TNF-α 3M	15.548	1.102	.284	14.937	16.158	54.601	14	.000

TABLE 14

GROUP-II

Variables	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval				
				Lower	Upper			
GBI BL - GBI 3M	62.692	5.468	1.411	59.663	65.720	44.404	14	.000
PPD BL - PPD 3M	2.766	.479	.123	2.500	3.031	22.331	14	.000
CAL BL - CAL 3M	2.765	.474	.122	2.502	3.028	22.551	14	.000
PI BL - PI 3M	1.302	.242	.062	1.168	1.436	20.835	14	.000
TNF-α BL - TNF-α 3M	15.164	1.135	.293	14.535	15.793	51.702	14	.000

COMPARISON OF CLINICAL PARAMETERS BETWEEN CONTROL AND STUDY GROUPS

UNPAIRED T TEST

TABLE 15

BASELINE

Variables	Differences				t	df	Sig. (2-tailed)
	Mean	Std. Error Mean	95% Confidence Interval				
			Lower	Upper			
G-I GBI BL – G-II GBI BL	3.150	2.350	1.665	7.965	1.339	28	0.095
G-I PPD BL - G-II PPD BL	.803	.389	.005	1.601	2.062	28	0.024
G-I CAL BL - G-IICAL BL	.802	.390	.001	1.602	2.053	28	0.024
G-I PI BL – G-II PI BL	.016	.143	.278	.311	0.115	28	0.454

TABLE 16

3 MONTHS

Variables	Differences				t	df	Sig. (2-tailed)
	Mean	Std. Error Mean	95% Confidence Interval				
			Lower	Upper			
G-I GBI 3M - G-II GBI 3M	1.326	2.092	2.960	5.612	0.633	28	0.265
G-I PPD 3M - G-II PPD 3M	.403	.262	.133	.940	1.538	28	0.067
G-I CAL 3M - G-II CAL 3M	.419	.288	3.601	4.782	14.549	28	.000
G-I PI 3M - G-II PI 3M	.020	.077	.137	.178	0.267	28	0.395

CORRELATION BETWEEN CLINICAL PARAMETERS AND TNF-α LEVEL AT BASELINE AND 3MONTHS AFTER PHASE-I THERAPY

PEARSON CORRELATION

GROUP-I

TABLE 17

CLINICAL PARAMETERS		TNF-α
BASELINE GBI	Pearson Correlation	-.483
	Sig. (2-tailed)	.068
BASELINE PPD	Pearson Correlation	.990
	Sig. (2-tailed)	.000
BASELINE CAL	Pearson Correlation	.990
	Sig. (2-tailed)	.000
BASELINE PI	Pearson Correlation	.416
	Sig. (2-tailed)	.123

TABLE 18

CLINICAL PARAMETERS		TNF-α
3 MONTHS GBI	Pearson Correlation	-.280
	Sig. (2-tailed)	.313
3 MONTHS PPD	Pearson Correlation	.677
	Sig. (2-tailed)	.006
3 MONTHS CAL	Pearson Correlation	.682
	Sig. (2-tailed)	.005
3 MONTHS PI	Pearson Correlation	.073
	Sig. (2-tailed)	.795

CORRELATION BETWEEN CLINICAL PARAMETERS AND TNF-α LEVEL AT BASELINE AND 3MONTHS AFTER PHASE-I THERAPY

PEARSON CORRELATION

GROUP-II

TABLE 19

CLINICAL PARAMETERS		TNF-α
BASELINE GBI	Pearson Correlation	-.027
	Sig. (2-tailed)	.923
BASELINE PPD	Pearson Correlation	.970
	Sig. (2-tailed)	.000
BASELINE CAL	Pearson Correlation	.970
	Sig. (2-tailed)	.000
BASELINE PI	Pearson Correlation	-.357
	Sig. (2-tailed)	.191

TABLE 20

CLINICAL PARAMETERS		TNF-α
3 MONTHS GBI	Pearson Correlation	-.075
	Sig. (2-tailed)	.790
3 MONTHS PPD	Pearson Correlation	.916
	Sig. (2-tailed)	.000
3 MONTHS CAL	Pearson Correlation	.920
	Sig. (2-tailed)	.000
3 MONTHS PI	Pearson Correlation	-.179
	Sig. (2-tailed)	.523

COMPARISON OF SALIVARY TNF-α LEVEL BETWEEN GROUP-I AND GROUP-II

TABLE 21

AT BASELINE

Variables	Differences				t	df	Sig. (2-tailed)
	Mean	Std. Error Mean	95% Confidence Interval				
			Lower	Upper			
G-I TNF-α BL vs. G-II TNF-α BL	9.730	1.330	7.005	12.456	7.313	28	.000

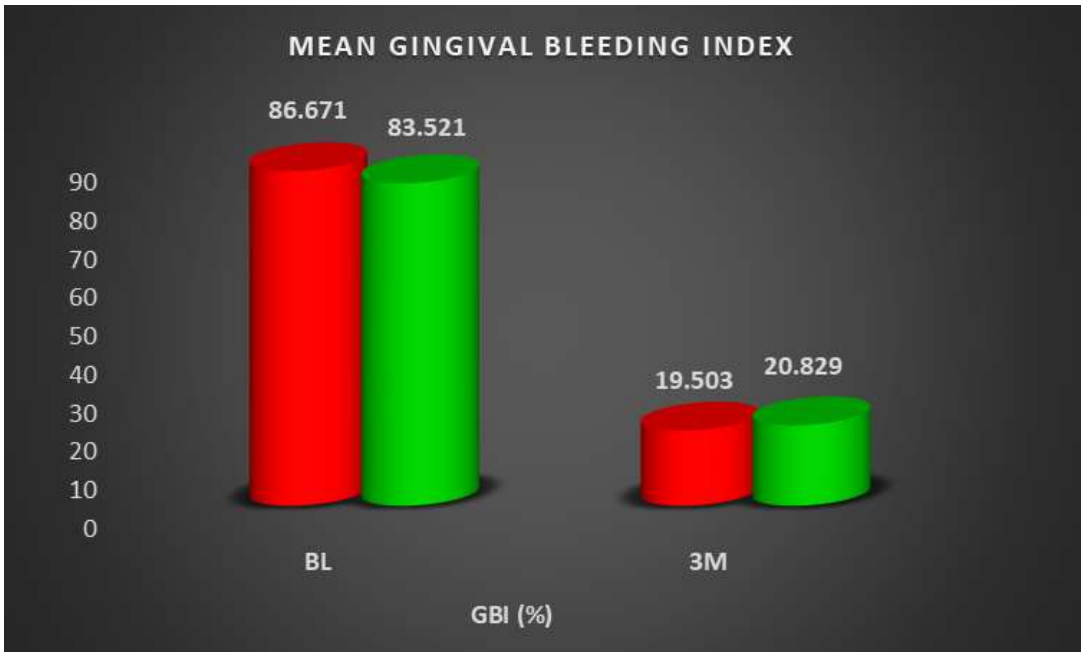
TABLE 22

AT 3MONTHS

Variables	Differences				t	df	Sig. (2-tailed)
	Mean	Std. Error Mean	95% Confidence Interval				
			Lower	Upper			
G-I TNF-α 3M vs. G-II TNF-α 3M	10.114	.925	8.218	12.009	10.931	28	.000

BAR DIAGRAM - 1

COMPARISON OF MEAN GINGIVAL BLEEDING INDEX BETWEEN GROUP-I AND GROUP-II AT BASELINE AND 3MONTHS AFTER PHASE I THERAPY

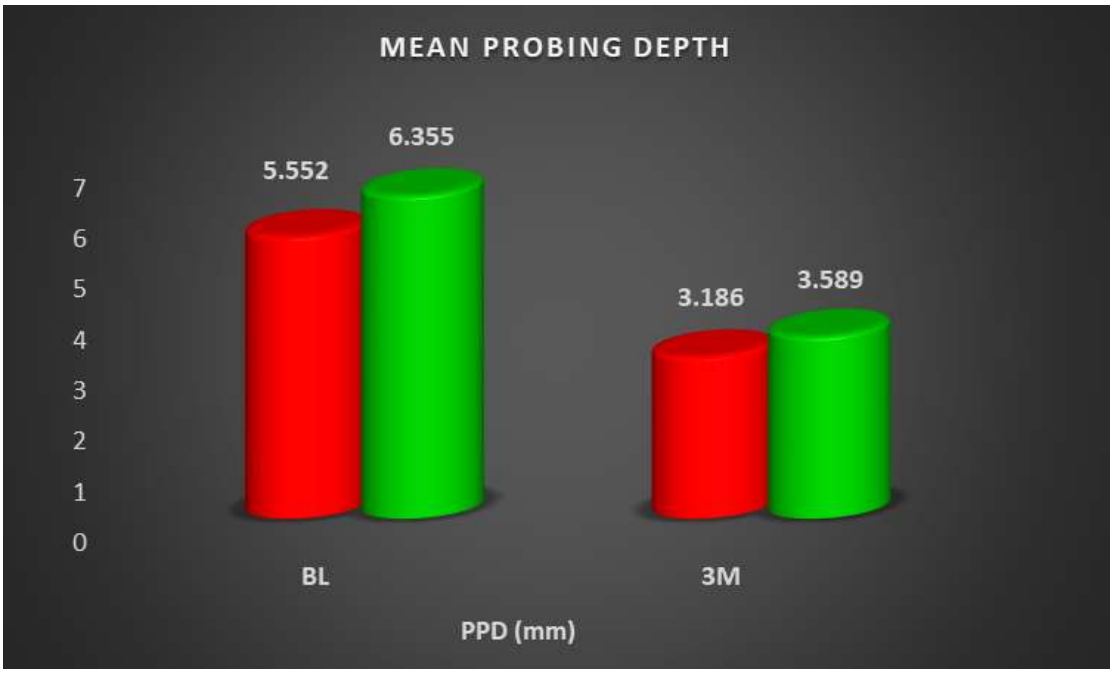


GROUP-I (CONTROL GROUP)

GROUP-II (STUDY GROUP)

BAR DIAGRAM - 2

COMPARISON OF MEAN PROBING POCKET DEPTH BETWEEN GROUP-I AND GROUP-II AT BASELINE AND 3MONTHS AFTER PHASE I THERAPY

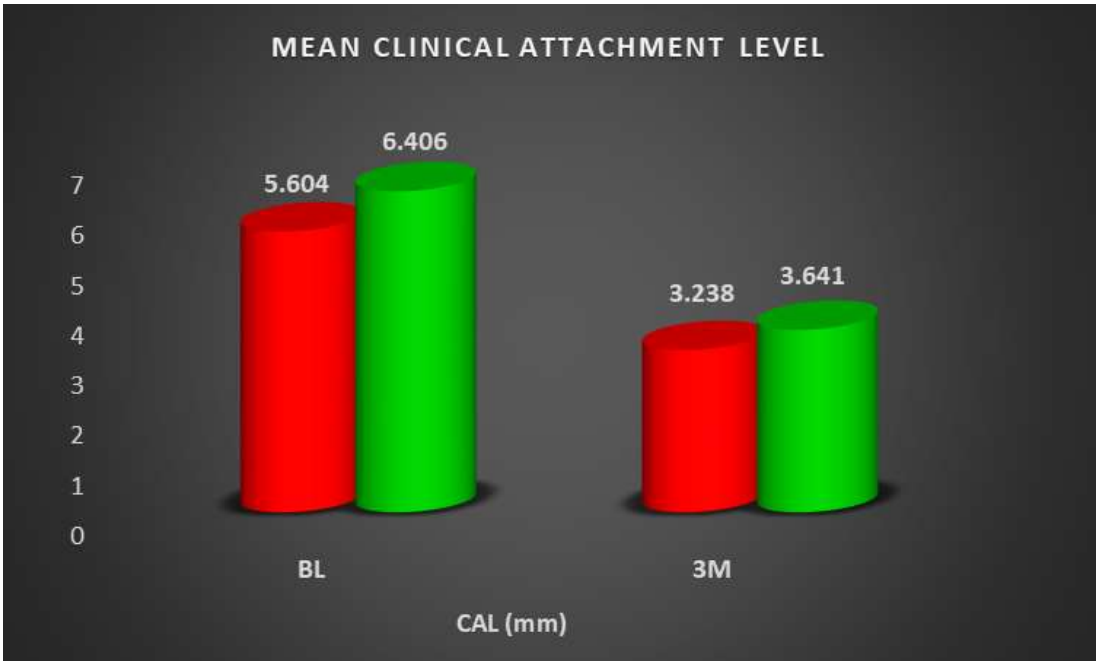


GROUP-I (CONTROL GROUP)

GROUP-II (STUDY GROUP)

BAR DIAGRAM - 3

COMPARISON OF MEAN CLINICAL ATTACHMENT LEVEL BETWEEN GROUP-I AND GROUP-II AT BASELINE AND 3MONTHS AFTER PHASE I THERAPY

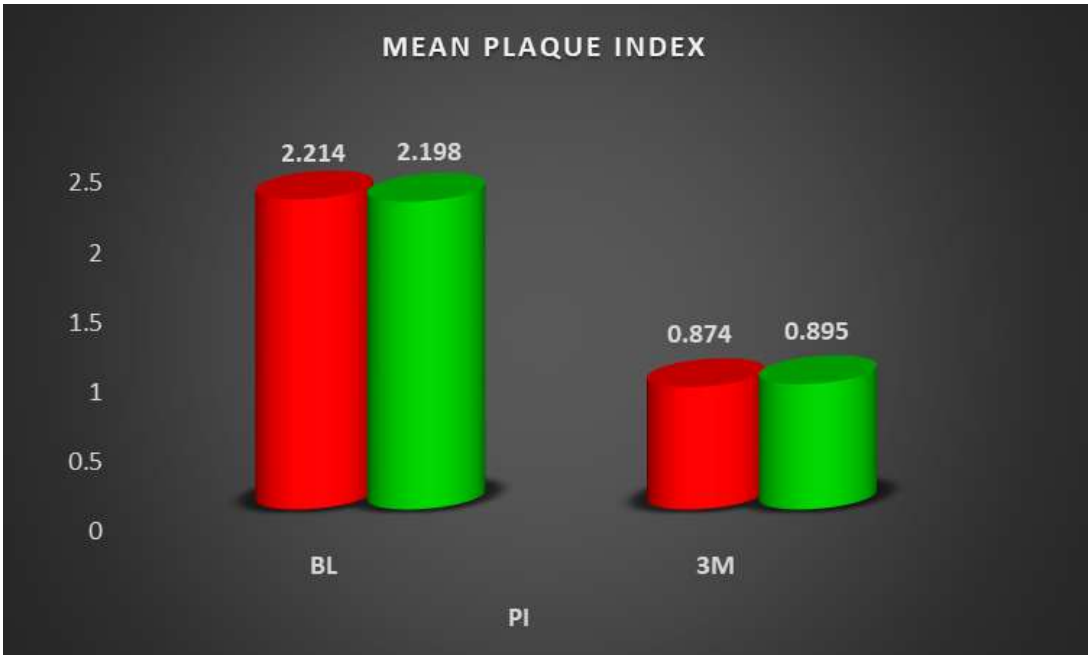


GROUP-I (CONTROL GROUP)

GROUP-II (STUDY GROUP)

BAR DIAGRAM - 4

COMPARISON OF MEAN PLAQUE INDEX BETWEEN GROUP-I AND GROUP-II AT BASELINE AND 3MONTHS AFTER PHASE I THERAPY

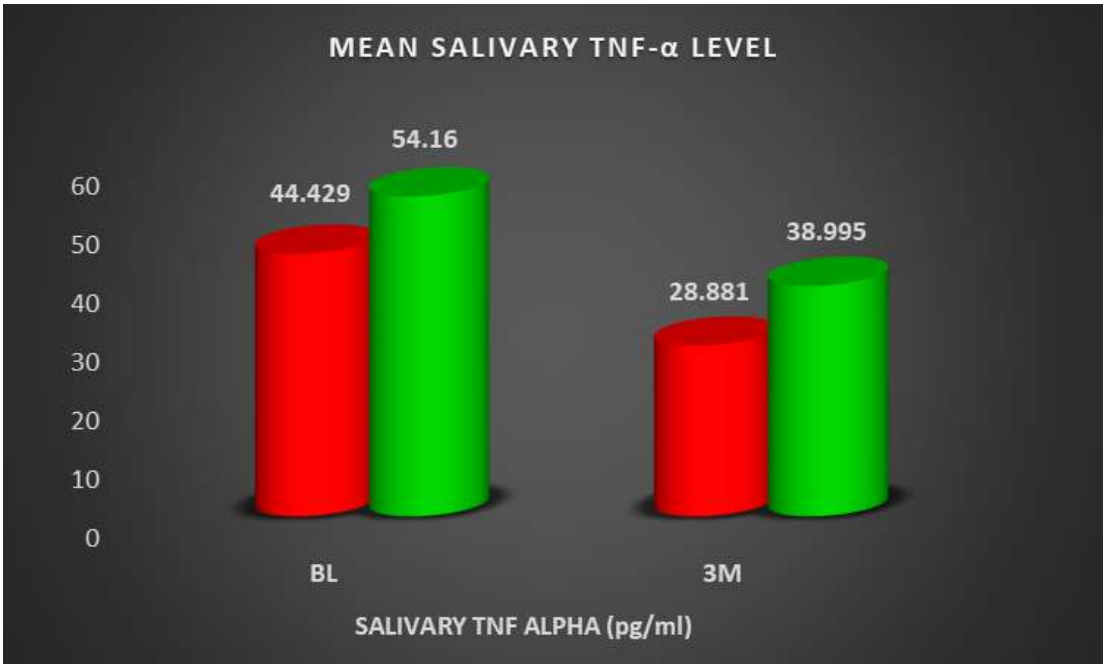


GROUP-I (CONTROL GROUP)

GROUP-II (STUDY GROUP)

BAR DIAGRAM - 5

COMPARISON OF MEAN SALIVARY TNF-α LEVEL BETWEEN GROUP-I AND GROUP-II AT BASELINE AND 3MONTHS AFTER PHASE I THERAPY



GROUP-I (CONTROL GROUP)

GROUP-II (STUDY GROUP)

DISCUSSION

Periodontitis is an inflammatory disease of the supporting tissues surrounding the teeth caused by heterogenous group of microorganisms, resulting in progressive destruction of the periodontal ligament, which if left untreated will progress to alveolar bone destruction ultimately resulting in tooth loss **(Marugame et al 2003)**[101].

An "inappropriate" cytokine milieu into periodontal tissues is thought to be one of the pathophysiological pathways that lead to destruction of periodontal tissues[15]. This is also thought to be an important biological mechanism through which periodontal diseases mediates its systemic effects. Inappropriate cytokine release is generally believed to be a result of an imbalance between destructive proinflammatory and protective anti-inflammatory cytokines. Smoking has been found to be associated with increased TNF-α level, thereby affecting the host response as well as the extent and severity of periodontal disease.

The primary objective of periodontal treatment is to preserve the dentition. Ideally, periodontal therapy should resolve inflammation, arrest disease progression, regenerate lost periodontium and create an environment that deters recurrent disease. To accomplish these goals both surgical and non-surgical procedures are employed. Various clinical trials done to evaluate the efficacy of non-surgical therapy have indicated that scaling and root planing can arrest the progression of periodontitis **(Badersten et al 1987, Philstrom et al 1981, 1984, 1989)**[102-105]. After scaling and root planing, investigators reported decreased gingival inflammation and less bleeding upon probing in patients with chronic periodontitis **(Singletary et al 1982, Greenewell et al 1984, Lavanchy et al 1987)**[106-108].

The use of saliva as a diagnostic medium has attracted much attention over the decades as it is a non-invasive medium containing an array of protein biomarkers. However the lack of site specificity and greater inter individual variability were thought to be its disadvantage as a diagnostic medium.

This study was hence undertaken to evaluate and compare the level of proinflammatory cytokine TNF-α in saliva of non-smokers and smokers with generalized chronic periodontitis before and after non-surgical phase-I therapy.

In the present study, subjects with any acute or chronic inflammatory conditions were excluded from the study because these conditions can affect the level of TNF-α, which may lead to confounding effect in the study. Patients under medications like antibiotics, corticosteroids or anti-inflammatory drugs for the past 3 months and those who underwent periodontal therapy within the past six months have also been excluded because these therapies can suppress the inflammatory process and may lead to confounding effect in the study.

In the present study two groups with 15 patients each having chronic periodontitis were evaluated. In group I, the patients were non-smokers while in group II, the patients were smokers. Phase-I periodontal therapy was done for the patients in both the groups. Clinical parameters like gingival bleeding index, plaque index, pocket probing depth and clinical attachment level were measured for each group at baseline and 3 months after therapy. Also comparison of salivary TNF-α level before and after therapy was done between the two groups.

Bleeding on probing is a commonly used diagnostic criterion for periodontal diseases. In this study, mean gingival bleeding index score (%) was 86.671±1.997 at baseline which reduced to 19.503±1.652 after phase-I therapy in group-I and 83.521±1.239 at baseline which reduced to 20.829±1.284 after phase-I therapy in

group-II. The mean gingival bleeding index score was less in smokers compared to non-smokers at baseline. This could be due to the vasocontrictive effect of smoking on the gingival blood vessels resulting in less gingival redness and bleeding on probing[66].

Mean difference in reduction of gingival bleeding index in group-I was 67.168±0.715 which was statistically significant (p=0.000) and in group-II was 62.629±1.411 which was also statistically significant (p=0.000). Within the limits of this study, these results proved that phase-I therapy produced significant outcome in the reduction of gingival bleeding. Also, the mean reduction in gingival bleeding index score after phase-I therapy was more in non-smokers compared to smokers. These results were in accordance with the results obtained in the study conducted by **Apatzidou et al** (2005)[109] assessing the impact of smoking on clinical indices. The sustained reduction in bleeding on probing is a reasonable prognostic indicator of periodontal health.

Probing pocket depth and clinical attachment level measurements are of prime importance in evaluating the success of a periodontal therapy. In this study, the mean probing pocket depth (mm) in group-I was 5.552±0.283 at baseline and 3.186±0.189 at 3 months postoperative review and in group-II it was 6.355±0.267 at baseline which reduced to 3.589±0.180 at 3 months after phase-I therapy. The mean clinical attachment level (mm) at baseline was 5.604±0.283 in group-I which reduced to 3.238±0.192 after phase-I therapy and 6.406±0.268 at baseline which reduced to 3.641±0.182 after phase-I therapy in group-II. The mean probing pocket depth and clinical attachment level were significantly higher in smokers compared to non-smokers at baseline. This could be explained due to impaired chemotaxis and phagocytosis and increased secretion of proteolytic enzymes that occurs as a result of smoking[66].

The mean difference in reduction of probing pocket depth in group-I was 2.366±0.214 and group-II was 2.766±0.123, both of which were statistically significant (p=0.000). The mean difference in reduction of clinical attachment level in group-I was 2.366±0.213 and group-II was 2.765±0.122, both of which were statistically significant (p=0.000). Within the limits of the present study, it is evident that phase-I therapy produced significant outcome in the reduction of probing pocket depth and clinical attachment level. Also, non-smokers showed better response to phase-I therapy compared to smokers as evident by increased reduction in probing pocket depth and clinical attachment level in group-I. These results were also comparable to the study conducted by **Apatzidou et al** (2005)[109] to assess the impact of smoking on clinical indices.

The mean plaque index in group-I was found to be 2.214±0.103 at baseline which reduced to 0.874±0.056 3months after phase-I therapy. In group-II it was 2.198±0.099 at baseline and reduced to 0.895±0.052 at 3months postoperative review. The mean difference in reduction of plaque index was 1.340±0.075 in group-I and 1.302±0.062 in group-II both of which were found to be statistically significant. This signifies that phase-I therapy resulted in significant improvement in oral hygiene.

TNF-α up regulates the expression of RANKL and thereby leading to increased osteoclastogenisis and contributes to the increased bone loss seen in periodontal diseases[103]. It is also responsible for the connective tissue destruction as it mediates the release of collagenase and degradation of type 1 collagen by fibroblasts[110]. Considering the key role this cytokine plays in mediating the disease process, the significant increase observed in periodontitis samples was along expected lines. The results of the present study were in accordance with **Frodge et al**[92], who found an increase in the levels of salivary TNF-α in chronic periodontitis patients compared to health.

The mean salivary TNF-α level in group-I was found to be 44.429±0.817 at baseline which reduced to 28.881±0.533 at 3months after phase-I therapy and in group-II it was 54.160±1.049 at baseline and reduced to 38.995±0.756 3months after phase-I therapy. The mean difference of baseline values of salivary TNF-α level between group-I and II was 9.730±1.330 which was statistically significant (p=0.000). The mean difference of 3months postoperative value was 10.114±0.925 which was also statistically significant (p=0.000). The mean salivary TNF-α level at baseline was found to be higher in smokers compared to non-smokers.

The mean reduction in mean salivary TNF-α level in group-I was 15.548±0.284 which was statistically significant (p=0.000) and in group-II it was 15.164±0.293 which was also statistically significant (p=0.000). This implies that phase-I therapy produced significant reduction in salivary TNF-α level and non-smokers showed greater reduction in salivary TNF-α level compared to smokers. The result obtained in the present study was also concurrent to the results of **Bostrom et al** (1991)[111] in which TNF-α level was elevated in GCF of smokers compared to non-smokers and non-smokers showed better response to treatment.

Correlation between clinical parameters and TNF-α level was obtained using Pearson correlation. In group-I, statistically significant positive correlation with salivary TNF-α level was found with baseline probing pocket depth (Pearson correlation=0.990, p=0.000) and baseline clinical attachment level (Pearson correlation=0.990, p=0.006) as well as with 3 months postoperative probing pocket depth (Pearson correlation=0.677, p=0.000) and clinical attachment level (Pearson correlation=0.682, p=0.005).

In group-II, statistically significant positive correlation with salivary TNF-α level was found with baseline probing pocket depth (Pearson correlation=0.970, p=0.000) and baseline clinical attachment level (Pearson correlation=0.970, p=0.006) as well as with 3 months postoperative probing pocket depth (Pearson correlation=0.677, p=0.000) and clinical attachment level (Pearson correlation=0.682, p=0.005).

Taking together, the results of the present study indicate that salivary markers need to be interpreted with a lot of caution as they may not accurately reflect serum levels. However, salivary cytokines may be used to detect periodontal disease with reasonable accuracy as there was a statistically significant difference obtained in the TNF alpha levels.

It must be clearly understood that periodontal diseases involve interaction of various cytokines that take part in the inflammatory process and environmental factors like smoking can affect the level of these cytokines and potentiate periodontal disease progression. Hence analysis of a few cytokines would not depict the progression of inflammatory process in the periodontal tissues. Therefore an array of other cytokines that are released into the periodontal environment should also be analyzed to map the pathogenesis that leads to periodontal diseases. Thus a panel of salivary cytokines may provide accurate prediction of the periodontal disease activity and may be used for diagnostic and prognostic purposes.

The cross sectional study design may minimize the strength of evidence as the limited samples size may not represent the entire population from where they are drawn, may provide differing results if another time frame is chosen and is prone for Neyman bias. Hence further studies are required to asses multiple cytokine profile in periodontal

diseases to predict the progression of diseases and therapeutic outcome of the periodontal intervention.

Within the limitations of the present study, these results demonstrate the value of salivary diagnostics in periodontal disease and the positive association between smoking and TNF-α level in chronic periodontitis.

SUMMARY AND CONCLUSION

The aim of the present study was to estimate salivary levels of the proinflammatory cytokine TNF-α in non-smokers and smokers with generalized chronic periodontitis before and after phase-I periodontal therapy and to correlate the salivary TNF-α level with clinical parameters such as gingival bleeding index, probing pocket depth, clinical attachment level and plaque index.

From the results of this study, the following conclusions could be drawn:

1. Elevated baseline level of salivary TNF-α level in smokers with chronic periodontitis compared to non-smokers with chronic periodontitis.

2. Significant reduction in the levels of clinical parameters after phase-I periodontal therapy in both group I and group II.

3. Significant reduction in the level of salivary TNF-α level after phase-I periodontal therapy in both group I and group II.

4. Non-smokers experienced significantly greater reduction in clinical parameters and salivary TNF-α level in response to phase-I therapy compared to smokers.

Within the limits of this study, it can be concluded that salivary TNF-α level can be considered as a biomarker for diagnosis, evaluation of treatment response and prognosis of generalized chronic periodontitis. Importance has been given to salivary TNF-α as a potential diagnostic aid, however further long term studies with greater sample size are required to confirm these results and to investigate whether salivary TNF-α estimation can be used as a chair side diagnostic aid for detection of periodontitis in a clinical setting.

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PARTICIPANT INFORMATION SHEET

INVESTIGATOR: DR. M.AADHIRAI GUIDED BY: DR. K.MALATHI

TITLE OF THE STUDY:

A comparative study on salivary Tumor Necrosis Factor-alpha levels in non-smokers and smokers with Chronic Periodontitis before and after Phase-I Periodontal therapy.

NAME OF THE RESEARCH INSTITUTION:

Tamil Nadu Government Dental College and Hospital, Chennai.

PURPOSE OF THE STUDY:

The purpose of this study is to compare the levels of Tumor Necrosis Factor-alpha in the saliva of non-smokers and smokers with chronic periodontitis before and after phase-1 periodontal therapy.

PROCEDURE:

Saliva sample will be collected by noninvasive method from all the subjects before and after treatment.

Routine blood investigations will be done for all the subjects before performing phase-I therapy.

Intra oral examination will be done and clinical parameters like bleeding index, plaque index, probing pocket depth and clinical attachment level will be estimated.

0.5ml of local anaesthetic solution will be injected as test dose. local anaesthesia will then be administered at diseased sites and scaling and root planning will be done using ultrasonic scaler and hand instruments.

RISK OF PARTICIPATION:

Radiation exposure during IOPA view radiograph procedure. Pain and discomfort due to scaling and root planning. This will be informed to the patients and necessary precautions will be taken.

- Lead apron and thyroid collars will be used while taking radiographs.

- All instruments will be of standard quality and sterile.
- Analgesics will be prescribed after treatment if necessary.
- If requires to be prescribed any medications for their general illness during the three months interval between sample collection, they will be excluded from the study.

BENEFITS OF PARTICIPATION:

Scaling and root planning will be done for improvement of periodontal health and patients will be educated about the harmful effects of smoking to help in cessation of the habit.

CONFIDENTIALITY:

The identity of the patients participating in the study will be kept confidential throughout the study. In the event of publication or presentation resulting from research, no personally identifiable information will be shared. Each participant will be given separate identity number, to be identified by their ID number and not by their names thereafter during the period of the study.

PARTICIPANTS RIGHTS:

Taking part in the study is voluntary. You are free to decide whether to participate in the study or to withdraw at any time. Your decision will not result in any loss of benefits to which you are otherwise entitled.

COMPENSATION: Nil

_____ _____

Name of the participant **Signature of the participant**

For queries related to study:
Dr.M.Aadhirai
No.105, 6th cross street,
Thirumalai nagar,
Perungudi,
Chennai-600096.

For queries related to rights of participant:
Dr.P.Saravanan,MDS
The Chairperson,
Institutional Ethical Committee,
Tamilnadu Government Dental College and Hospital, Chennai-600003.

ஆராய்ச்சி பற்றிய தகவல் படிவம்

ஆராய்ச்சி மேற்கௌள்பவர்: **வழி நடத்துபவர்:**

மருத்துவர்.எம்.ஆதிரை
மருத்தவர்.கே.மாலதி

ஆராய்ச்சியின் தலைப்பு

"புகை பிடிக்கும் பழக்கமுடைய மற்றும் பழக்கம் இல்லாத ஈறு அழற்சி நோய் உடையவர்களின் உமிழ் நீரில் உள்ள டிஎன்எஃப்-ஆல்பா வின் அளவை அறுவை சிகிச்சையில்லா ஈறு மருத்துவத்திற்கு முன்பும் பின்பும் ஒப்பீடு செய்யும் ஆராய்ச்சி"

செய்முறை:

கீழ்கண்ட ஆய்வுகள்/பரிசோதனைகள் உங்களுக்கு செய்யப்படும்:

- வாய் பரிசோதனை - உட்புறம், வெளிப்பறம்.
- வழக்கமான இரத்தப் பரிசோதனை செய்யப்படும்.
- ஓவ்வாமை ஏற்படுகிறதா என்பதை தெரிந்துகொள்ள 0.5மிலி 2% லிக்னோகெயின் என்னும் மரத்துப்போக செய்யும் மருந்து உங்களின் கையில் பரிசோதனைக்காக செலுத்தப்படும். பின்பு நோயுற்ற பகுதியில் இம்மருந்து செலுத்தப்படும்.
- நோயுற்ற பகுதியின் ஊடுகதிர் படம் எடுக்கப்படும்.
- அல்ட்ரா சோனிக் ஸ்கேலர் மற்றும் கைக்கருவிகள் பயன்படுத்தி பல் மற்றும் பல்லின் வேர் சுத்தம் செய்ப்படும். உப்பு நீர் கொண்டு நோயுற்ற பகுதி சுத்தம் செய்யப்படும்.
- சிகிச்சைக்கு முன்பும் பின்பம் ஆராய்ச்சிக்காக தங்களின் உமிழ் நீர் சேகரிக்கப்படும்.

பங்கேற்பதினால் வரக்கூடிய பக்க விளைவுகள்:

பக்க விளைவுகள் ஏற்படாமல் தடுக்க உரிய முறைகள் பின்பற்றப்படும்.

- ஊடுகதிர் படம் எடுக்கப்படும் பொழுது லெட் ஏப்ரன், தைராய்டு காலர் போன்ற பாதுகாப்பு உபகரணங்கள் பயன்படுத்தப்படும்.
- சிறந்த தரம் மற்றும் சுத்தமான கருவிகள் பயன்படுத்தப்படும்.
- சிகிச்சைக்குப்பின் தேவைபட்டால் வலிநிவாரண மருந்துகள் கொடுக்கப்படும்.

<u>பங்கேற்பதினால் விளையும் நன்மைகள்:</u>

உங்களின் ஈறு அழற்சி நோய்க்கு அறுவை சிகிச்சையில்லா ஈறு மருத்துவமுறையின்படி சிகிச்சை அளிக்கப்படும்.புகை பிடிப்பதினால் விளையம் தீமைகள் எடுத்துரைக்கப்படும்.

<u>இரகசிய காப்பு:</u>

உங்களை பற்றிய குறிப்புகள் பிறர் அறியா வண்ணம் ஆராய்ச்சி முடியும் வரை இரகசியமாக பாதுகாக்கப்படும். அதை வெளிப்படுத்தும் நேரங்களிள் எந்த தனிநபர் அடையாளங்களும் வெளிப்பட வாய்ப்பு கிடையாது.

<u>தன்னார்வ பங்கேற்பு:</u>

இந்த ஆராய்ச்சியில் பங்குபெறுவது தங்களின் தனிப்பட்ட முடிவு மற்றும் இந்த ஆராய்ச்சியிலிருந்து தாங்கள் எப்பொழுது வேண்டுமானாலும் விலகிக்கொள்ளலாம்.தங்களின் இந்த திடீர் முடிவு தங்களுக்கோ அல்லது ஆராய்ச்சியாளருக்கோ எவ்வித பாதிப்பம் ஏற்படுத்தாது என்பதை தெரிவித்துக்கொள்கிறோம்.

_____	_____
நோயாளியின் பெயர்	நோயாளியின் கையொப்பம்

ஆராய்ச்சி தொடர்புடைய தகவல்களுக்கு:

மருத்துவர்.எம்.ஆதிரை,
எண்.105, 6வது குறுக்குத் தெரு,
திருமலை நகர், பெருங்குடி,
சென்னை-600096.

பங்கேற்பாளரின் உரிமை தொடற்புடைய தகவல்களுக்கு:
மருத்துவர்.பி.சரவணனன்,
தலைவர்,
நிறுவன நெறிமுறைகள் குழு,
தமிழ்நாடு அரசு பல் மருத்துவமனை மற்றும் கல்லூரி, சென்னை-600003.

INFORMED CONSENT FORM

"A COMPARATIVE STUDY ON SALIVARY TUMOR NECROSIS FACTOR-ALPHA LEVELS IN NON-SMOKERS AND SMOKERS WITH CHRONIC PERIODONTITIS BEFORE AND AFTER PHASE-I PERIODONTAL THERAPY"

"I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this study and understand that I have the right to withdraw from the study at any time without in any way it affecting my further medical care."

_____	_____	_____
Date	Name of the participant	Signature/Thumb impression of the participant

[The literate witness selected by the participant must sign the informed consent form. The witness should not have any relationship with the research team; If the participant does not want to disclose his/her participation details to others, in view of respecting the wishes of the participant, he/she can be allowed to waive from the witness procedure. (This is applicable to literate participant ONLY). This should be documented by the study staff by getting signature from the prospective participant.]

"I have witnessed the accurate reading of the consent form to the potential participant and the individual has had opportunity to ask questions. I confirm that the individual has given consent freely."

_____	_____	_____
Date	Name of the witness	Signature of the witness
_____	_____	_____
Date	Name of the interviewer	Signature of the interviewer

ஆராய்ச்சி ஒப்புதல் படிவம்

ஆராய்ச்சி தலைப்பு

“புகை பிடிக்கும் பழக்கமுடைய மற்றும் பழக்கம் இல்லாத ஈறு அழற்சி நோய் உடையவர்களின் உமிழ் நீரில் உள்ள டிஎன்எஃப்-ஆல்பா வின் அளவை அறுவை சிகிச்சையில்லா ஈறு மருத்துவத்திற்கு முன்பும் பின்பும் ஒப்பீடு செய்யும் ஆராய்ச்சி”

பெயர்: **வயது/பால்:**

முகவரி: **தொலைபேசி:**

புற நோயாளி எண்:

ஆராய்ச்சி சேர்க்கை எண்:

நான் _____ வயது _____ என்னுடைய சுய நினைவுடனுன் மற்றும் முழு சுதந்திரத்திரத்துடனும் இந்த மருத்துவ ஆராய்ச்சியில் என்னை சேர்த்துக்கொள்ள ஒப்புதல் அளிக்கிறேன்.

கீழ்காணப்படும் நிபந்தனைகளுக்கு நான் சம்மதிக்கிரேன்:

நான் இந்த ஆராய்ச்சியின் நோக்கம் மற்றும் செய்முறைகள் பற்றி முழுமையாக தெரிவிக்கப்பட்டுள்ளேன்.

இந்த பரிசோதனைக்காக பற்களை சுத்தம் செய்ய வேண்டியுள்ளதாக அறிகிறேன்.

என் உடல் நலம் பாதிக்கப்பட்டாளோ அள்ளது எதிர்பாராத வழகத்திற்கு மாறான நோய்குறிகள் தென்பட்டாளோ அதற்கு சிகிச்சை பெற்றுகொள்வதற்கும் முழு உரிமை உள்ளதாக அறிகிறேன்.

நான் எற்கனவே உட்கொண்ட மற்றம் உட்கொள்கின்ற மருந்துகளின் விபரங்களை ஆராய்சியாளரிடம் தெரிவித்துள்ளேன்.

என் மருத்துவ குறிப்பேடுகளை இந்த ஆராய்ச்சியிள் பயன்படுத்திக்கொள்ள சம்மதிக்கிரேன்.

இந்ந ஆராய்ச்சி மையமும் ஆராய்ச்சியாளரும் என்னுடைய விபரங்கள் அனைத்தயும் இரகசியமாக வைப்பதாக அறிகிறேன்.

_____	_____	_____
நோயாளியின் பெயர்	**கையொப்பம்**	**தேதி**
_____	_____	_____
ஆராய்ச்சியாளரின் பெயர்	**கையொப்பம்**	**தேதி**

PROFORMA

DEPARTMENT OF PERIODONTICS

TAMILNADU GOVERNMENT DENTAL COLLEGE AND HOSPITAL

CHENNAI – 600 003

"A COMPARATIVE STUDY ON SALIVARY TUMOR NECROSIS FACTOR-ALPHA LEVELS IN NON-SMOKERS AND SMOKERS WITH CHRONIC PERIODONTITIS BEFORE AND AFTER PHASE-I PERIODONTAL THERAPY"

PROFORMA

OP No: DATE: GROUP:

NAME: PATIENT ID No:

AGE/SEX:

ADDRESS:

MOBILE No:

OCCUPATION:

INCOME:

CHIEF COMPLAINT:

HISTORY OF PERSENTING ILLNESS:

PAST MEDICAL HISTORY:

PAST DENTAL HISTORY:

HISTORY OF HABITS:

INTRA ORAL EXAMINATION:

1) No. of teeth present:

2) Gingival Examination:

Colour:

Contour:

Consistency:

Texture:

Position:

Pigmentation:

3) Recession:

4) Mobility:

GINGIVAL BLEEDING INDEX

3																
B																
	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38
B																
3																

CALCULATION

BASELINE

SCORE: **INFERENCE:**

3 MONTHS

SCORE: **INFERENCE:**

PROBING POCKET DEPTH AND CLINICAL ATTACHMENT LEVEL

3	CAL																
	PPD																
B	CAL																
	PPD																
3	CAL																
	PPD																
B	CAL																
	PPD																
		18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
		48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38
B	PPD																
	CAL																
3	PPD																
	CAL																
B	PPD																
	CAL																
3	PPD																
	CAL																

PLAQUE INDEX – SILNESS AND LOE (1964)

B																
	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
3																
B																
	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38
3																

INVESTIGATIONS

1) Blood investigation

Hemoglobin count

Total leucocyte count

Differential leucocyte count

Bleeding time

Clotting time

Random blood sugar

2) Salivary Tumor Necrosis Factor-alpha level

SAMPLE	BASELINE	3 MONTHS
SALIVARY TNF-alpha		

DIAGNOSIS

PROGNOSIS

TREATMENT

PHASE-I:

S.NO	CALCULATIONS	BASELINE	3 MONTHS
1	Plaque index		
2	Gingival bleeding index		
3	Probing pocket depth		
4	Clinical attachment level		
5	Salivary TNF-alpha		

SIGNATURE OF PG STUDENT SIGNATURE OF GUIDE

DATE